U.S. DEPARTMENT OF COMMERCE
National Technical Information Service

PB-280 115

Toxicology of Metals
Volume III

Permanent Commission and Internat'l Assn. of Occupational Health

Prepared for

Health Effects Research Lab, Research Triangle Park, N C

Feb 78

EPA-600/1-78-016
February 1978

Environmental Health Effects Research Series

PB 280 115

TOXICOLOGY OF METALS - Volume III

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U. S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

Health Effects Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Research Triangle Park, North Carolina 27711

308

RESEARCH REPORTING SERIES

Research reports of the Office of Research and Development, U.S. Environmental Protection Agency, have been grouped into nine series. These nine broad categories were established to facilitate further development and application of environmental technology. Elimination of traditional grouping was consciously planned to foster technology transfer and a maximum interface in related fields. The nine series are:

1. Environmental Health Effects Research
2. Environmental Protection Technology
3. Ecological Research
4. Environmental Monitoring
5. Socioeconomic Environmental Studies
6. Scientific and Technical Assessment Reports (STAR)
7. Interagency Energy-Environment Research and Development
8. "Special" Reports
9. Miscellaneous Reports

This report has been assigned to the ENVIRONMENTAL HEALTH EFFECTS RESEARCH series. This series describes projects and studies relating to the tolerances of man for unhealthful substances or conditions. This work is generally assessed from a medical viewpoint, including physiological or psychological studies. In addition to toxicology and other medical specialities, study areas include biomedical instrumentation and health research techniques utilizing animals — but always with intended application to human health measures.

This document is available to the public through the National Technical Information Service, Springfield, Virginia 22161.

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA-600/1-78-016	2.	3. RECIPIENT'S ACCESSION NO. PB280115
4. TITLE AND SUBTITLE TOXICOLOGY OF METALS - Volume III		5. REPORT DATE February 1978 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Lars Friberg, Chairman		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Subcommittee on the Toxicology of Metals Permanent Commission and International Association of Occupational Health		10. PROGRAM ELEMENT NO. 1AA601 11. CONTRACT/GRANT NO. 68-02-1287
12. SPONSORING AGENCY NAME AND ADDRESS Health Effects Research Laboratory RTP,NC Office of Research and Development U.S. Enironmental Protection Agency Research Triangle Park, N.C. 27711		13. TYPE OF REPORT AND PERIOD COVERED 14. SPONSORING AGENCY CODE EPA 600/11

15. SUPPLEMENTARY NOTES

Prepared in cooperation with the Swedish Environmental Protection Board, and the Karolinska Institute

16. ABSTRACT

This report on metal toxicology contains reviews on the general aspects of metal toxicology. These have been written for inclusion in a Handbook on the Toxicology of Metals: Environmental and Occupational Aspects which is being prepared by the Scientific Committee on the Toxicology of Metals of the Permanent Commission and International Association of Occupational Health.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
metals toxicology reviews		06 T

18. DISTRIBUTION STATEMENT RELEASE TO PUBLIC	19. SECURITY CLASS *(This Report)* UNCLASSIFIED	21. NO. OF PAGES 308
	20. SECURITY CLASS *(This page)* UNCLASSIFIED	22. PRICE A14 - A01

EPA Form 2220-1 (9-73)

EPA-600/1-78-016
February 1978

TOXICOLOGY OF METALS - Volume III

by

Subcommittee on the Toxicology of Metals
Permanent Commission and International Association of
Occupational Health
Prof. Lars Friberg, Chairman

in cooperation with

The Swedish Environmental Protection Board, and
The Karolinska Institute

Contract No. 68-02-1287

Project Officer

Robert J. M. Horton
Criteria and Special Studies Office
Health Effects Research Laboratory
Research Triangle Park, N.C. 27711

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND DEVELOPMENT
HEALTH EFFECTS RESEARCH LABORATORY
RESEARCH TRIANGLE PARK, N.C. 27711

i.a

DISCLAIMER

This report has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the U.S. Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

TABLE OF CONTENTS

Page

Introduction 1

List of Contributors 3

GENERAL CHEMISTRY OF METALS
Velimir Vouk 5

SAMPLING AND ANALYTICAL METHODS
Magnus Piscator and Velimir Vouk 29

SOURCES, TRANSPORT, AND TRANSFORMATION OF METALS IN THE ENVIRONMENT
Karin Beijer and Arne Jernelov 51

EFFECTS - GENERAL PRINCIPLES UNDERLYING THE TOXIC ACTION OF METALS
Thomas W. Clarkson 83

FACTORS INFLUENCING EFFECTS AND DOSE-RESPONSE RELATIONSHIPS OF METALS
Gunnar Nordberg, Jiri Parizek and Magnus Piscator 117

EPIDEMIOLOGICAL ASPECTS OF ASSESSMENT OF DOSE-RESPONSE AND DOSE-EFFECT RELATIONSHIPS
Tord Kjellstrom 141

GENERAL ASPECTS OF THE PREVENTION OF METAL POISONING
Sven Hernberg 171

GENERAL ASPECTS AND SPECIFIC DATA ON ECOLOGICAL EFFECTS OF METALS
Karin Beijer and Arne Jernelov 201

STANDARDS AND CRITERIA
Lars Friberg and Velimir Vouk 223

CLINICAL EFFECTS, DIAGNOSIS AND TREATMENT OF METAL POISONING - GENERAL CONSIDERATIONS
George Kazantzis 237

MUTAGENIC AND CARCINOGENIC EFFECTS OF METALS
George Kazantzis and Lorna Lilly 265

INTRODUCTION

Introduction to Volume I

At the time of the XVIth International Congress on Occupational Health in Tokyo in 1969 the Permanent Commission and International Association of Occupational Health decided to form a subcommittee on the toxicology of metals. The need for such a group had been recognized during an international meeting on mercury toxicology in 1968. The subcommittee was formed with Prof. Lars Friberg of Stockholm as chairman. The program developed by this group consisted of exploration and documentation of the general principles of metal toxicology by means of symposia and workshops of experts in the field. A broad approach was selected including general environmental exposures as well as those related to occupation. Emphasis was placed on greater depth and precision of understanding in terms of metabolic processes and dose-effect relationships. The Permanent Commission, the Swedish Environmental Protection Board, and the Karolinska Institute have supported the committee in carrying out this program. Two workshops were conducted, one in conjunction with the meeting of the Permanent Commission in 1971, and another in conjunction with the XVIIth International Congress on Occupational Health in 1972.

Recently the subcommittee has decided to broaden its work by preparing a handbook on metal toxicology which will contain the general material being developed in its workshops and also specific information on a large number of metals for use by toxicologists and occupational and environmental health workers.

The Environmental Protection Agency is cooperating with the other sponsors in supporting this phase of the subcommittee's work. As information is developed on the subject it will be collected in three annual progress reports. The first of these is contained in this volume. It consists of the conclusions of the subcommittee's third workshop which was held in Tokyo in 1974. Since the three workshops which have been held constitute a cumulative series, with frequent reference back to previous definitions and statements, it was thought best on this occasion to reproduce all of them together as a unit.

Introduction to Volume III

This third volume on Toxicology of Metals contains material prepared for the Handbook of the Toxicology of Metals - Environmental and Occupational Aspects which is being prepared by the Scientific Committee on the Toxicology of Metals of the Permanent Commission and International Association of Occupational Health. Volume II contained chapters on the toxicology of specific metals. This third volume contains chapters on the general aspects of metal toxicity. Although these are finished chapters, it has occurred to the authors and editors that publication at this time offers the opportunity to solicit comments and suggestions from the scientific community which might be helpful in improving these texts before final publication in the handbook. Readers who know of additional pertinent material which should be included, or have other suggestions for improvements are urged to communicate directly as soon as convenient with the responsible senior authors whose addresses are given on page 3.

List of Contributors to this Volume

Karin Beijer, M.S.
The Swedish Water and Air Pollution Research Laboratory
Hälsingegatan 43
S-100 31 Stockholm, Sweden

Thomas W. Clarkson, Ph.D.
Department of Radiation Biology and Biophysics
School of Medicine and Dentistry
University of Rochester
Rochester, N.Y. 14627, U.S.A.

Lars Friberg, M.D.
Department of Environmental Hygiene
of The Karolinska Institute
and of The National Swedish Environment Protection Board
S-104 01 Stockholm 60, Sweden

Sven Hernberg, M.D.
Institute of Occupational Health
Haartmaninkatu 1
SF-00290 Helsinki 29, Finland

Arne Jernelöv, Ph.D.
The Swedish Water and Air Pollution Research Laboratory
Hälsingegatan 43
S-100 31 Stockholm, Sweden

George Kazantzis, M.D.
Department of Community Medicine
The Middlesex Hospital Medical School
Central Middlesex Hospital
London NW10 7NS, England

Tord Kjellström, M.D.
Department of Community Medicine
School of Medicine
University of Auckland
Private Bag
Auckland, New Zealand

Lorna Lilly, M.D.
Department of Biology as Applied to Medicine
Middlesex Hospital Medical School
Cleveland Street
London W 1, England

Gunnar Nordberg, M.D.
Institute of Community Health and Environmental Medicine
Odense University
Winslovsvej 19
DK-5000 Odense, Denmark

Jiri Parizek, M.D.
Institute of Physiology
Czechoslovak Academy of Sciences
Budejovicka 1083
CS-142 20 Prague 4
CSSR

Magnus Piscator, M.D.
Department of Environmental Hygiene
The Karolinska Institute
S-104 01 Stockholm 60, Sweden

Velimir Vouk, Ph.D.
Control of Environmental Pollution and Hazards
Division of Environmental Health
World Health Organization
1211 Geneva 27, Switzerland

Technical editor:

Pamela Boston, B.A.
Department of Environmental Hygiene
The National Swedish Environment Protection Board
S-104 01 Stockholm 60, Sweden

GENERAL CHEMISTRY OF METALS

Velimir Vouk

Out of 104 identified elements about 80 are usually considered as metals, and the chemistry of metals represents a major part of inorganic chemistry. The chemical basis of metal toxicology is still inadequately understood although significant advances have been made with the emergence of bio-inorganic chemistry.

For details on the chemistry of metals, the reader is referred to the recent texts on inorganic chemistry by Cotton and Wilkinson (1972, 1976). Those less familiar with modern chemistry are advised to consult the excellent introductory text by Pauling (1970). A good source of information on the properties of ions in solution, a field of great importance for the understanding of metal toxicology, is the introductory monograph by Parr (1973). The principles of bio-inorganic chemistry are discussed by Hughes (1972) and Phipps (1976) and some theoretical and chemical aspects of the toxicology of metals by Brakhanova (1975).

Most of the information contained in this chapter is based on the sources referred to above and, in view of its introductory character, original literature is not cited.

1. Definition of metals

Metals are usually defined on the basis of their physical properties in the solid state, which are of great technological significance. These are 1) high reflectivity which is responsible for the characteristic metallic luster, 2) high electrical conductance which decreases with increasing temperature, 3) high thermal conductance, and 4) mechanical properties such as strength and ductility. Metals are also characterized by their crystal structure, by their specific type of chemical bond in which the electrons are delocalized and mobile, and by their magnetic properties. Of particular technological usefulness are alloys, metallic materials consisting of two or more elements. These properties of metals have a limited interest for the understanding of systemic toxic effects, although some of them may be of importance as regards the local effects of metal aerosols.

From the toxicological viewpoint, a more useful definition of metals is that based on the properties of their ions in aqueous solutions: a metal is an element which under biologically significant conditions may react by losing one or more electrons to form a cation. Much of the discussion that follows will be concerned with the behavior of metal ions in solution.

The distinction between metals and non-metals, whether based on their physical or chemical properties, is not a sharp one. Some elements such as selenium or germanium have certain properties which are typical of metals; other properties make them similar to non-metals. This also applies to such elements as boron and silicon which have to be classified separately as metalloids or metal-like elements. In general, there is in some groups of the periodic system a gradual transition of properties from non-metals to metals as we descend from the lighter to the heavier elements (e.g. C, Si, Ge, Sn, Pb in the IVb group of the periodic system).

2. Classification of metals

The properties of chemical elements depend on the electronic configuration of the atom and vary with the atomic number in a systematic way. The periodicity of properties may be shown by arranging the elements in a periodic table (Table 1). The horizontal rows, called periods, consist of a very short period (containing hydrogen and helium), two short periods of 8 elements each, two periods of 18 elements each, a very long period of 32 elements, and an incomplete period. The vertical columns of the periodic table are the groups of elements. There are 9 groups of elements, groups I to VII being further subdivided into subgroups a and b.

The electronic configuration of elements is usually described in terms of shells, subshells and "orbitals" which indicate the spatial distribution of electrons. The so-called s-orbitals are spherically symmetrical around the nucleus of the atom. Other orbitals (p, d, etc.) are not spherically symmetrical (Figure 1). The first shell consists of one orbital (1s) which can accommodate at most two electrons, as indicated by the symbol $1s^2$. This is the electronic structure of the element helium. The second shell consists of two subshells (2s and 2p). The 2p subshell is further subdivided into 3 orbitals ($2p_x$, $2p_y$ and $2p_z$). Since each orbital can accomodate only 2 electrons (Pauli's exclusion principle), the second shell contains a maximum of 8 electrons. This is indicated by the symbol $2s^2\ 2p^6$. The element neon has both shells completely filled, and its electron structure is $(1s^2)\ (2s^2 2p^6)$. The next shell consists of 3 subshells: 3s (one orbital), 3p (three orbitals) and 3d (five orbitals). When completed this shell will accommodate a total of 18 electrons arranged as follows: $3s^2\ 3p^6\ 3d^{10}$. The fourth shell contains these subshells, and in addition a 6f subshell which consists of seven 4f orbitals. The filling of the successive shells, subshells and orbitals proceeds in the sequence of increasing energies which is, for the lighter elements, $1s<2s<2p<3s<3p<4s<3d<4p<4d<4f$.

According to their electronic configuration, metals are classified into s-, p-, d- and f-blocks (see Table 1). The s-block contains the alkali metals (Li, Na, K, Rb, Cs, Fr), beryllium (Be), magnesium (Mg) and the alkaline earths (Sr, Ba, Ra). Their electronic configuration is characterized by a noble gas core and one s (alkali metals) or two s electrons (Be, Mg and alkaline earths).

The d-block contains at least 32 elements which can be further subdivided into 3d and 4d and 5d metals. With the exception of Zn, Cd and Hg, the d-block metals belong to the so-called transition elements which have an incompletely filled d-orbital. The first series of transition elements (3d - sub-block) contains several biologically important metals such as vanadium, chromium, manganese, iron, cobalt and copper.

The f-block metals also belong to the transition elements. Their electronic configuration is characterized by partially filled 4f and 5f orbitals. The 4f elements are called lanthanides and have a very simple chemistry. In this respect they are similar to the s-block metals. The 5f metals(actinides) have a much more complex chemical behavior. They are the heaviest elements in the periodic table and are radioactive.

The p-block consists of 25 elements out of which only 9 are usually classified as metals (Al, Ga, In, Tl, Ge, Sn, Pb, Sb, Bi) although As, Se, Te and Po also exhibit some metallic properties.

3. Compounds of metallic elements

3.1 Covalent and ionic bonds

There are two extreme types of chemical bonds, covalent and ionic, and examples of each type of bond can be found among metal compounds. Since electrons are in continuous motion around the atomic nucleus, the positive charge of the nucleus, if averaged over all directions in space, is balanced by the negative charge of the electrons. However, if we select a specific direction in space, the positive charge of the nucleus will not be completely shielded by the electrons. Thus, if two atoms are brought together, the effective positive charge of each atom will displace the outer electrons (the valence electrons) of the other atom, so that the electron density becomes greater in the region between the two nuclei. In other words the two nuclei will share a pair of electrons. The resulting chemical bond is called covalent bond. Although quantum mechanical concepts such as pairing of electron spins are needed to describe a covalent bond, the basic binding force is the electrostatic interaction of the bonding electrons with the nuclei between which they are located. When the two atoms of a diatomic molecule are the same (e.g. two hydrogen atoms) the electron density is distributed symmetrically between the two nuclei and the covalent bond is homopolar. If the two atoms are not identical, the distribution of electrons between the two nuclei will be asymmetrical, and the electron density will be displaced towards the atomic nucleus which is more electronegative, i.e. which has a higher capacity to attract electrons. This is a heteropolar covalent bond. The greater the difference in electronegativities of the two atoms forming a bond, the more uneven will be the distribution of electrons between the two atoms, and an extreme case can be envisaged in which an electron has been entirely transferred from one atom to the other, forming a completely electrostatic or ionic bond. Metallic elements have a low electronegativity, and are relatively easily ionized by non-metallic elements with high electronegativity (e.g. chlorine), thus forming ionic bonds. The ionic bond is a useful model but it should be understood that a chemical bond is rarely either entirely covalent or entirely ionic. The electron distribution in homopolar, heteropolar and ionic bonds is shown in Figure 2.

Predominantly ionic bonds are found in metal salts such as NaCl or $Ca(NO_3)_2$. Predominantly covalent bonds are formed between metals and carbon atoms, for example in organometallic compounds such as dimethylmercury $CH_3 \cdot Hg \cdot CH_3$, but there are also other metal compounds with covalent bonds.

3.2 Inorganic compounds

Metallic elements form a variety of inorganic compounds. They may be classified into the binary compounds and multi-element compounds.

The most important binary compounds, both from the technological and toxicological viewpoint, are oxides and sulfides. This is the chemical form in which most metals appear in nature as minerals, and metal-containing aerosols produced in the course of metallurgical processes are usually metal oxides or sulfides. Closely related to sulfides are binary compounds of metals with the elements tellurium and selenium (tellurides and selenides).

Another class of important binary metal compounds consists of halides, i.e. compounds of metallic elements with fluorine, chlorine, bromine and iodine. Most metal halides are substances of predominantly ionic character although there is a gradation from purely ionic to essentially covalent compounds. For example, KCl is completely ionic but $TiCl_4$ is a covalent molecular compound.

Metallic elements can also form binary compounds with hydrogen (hydrides), carbon (carbides), and nitrogen (nitrides). All these compounds may be either predominantly ionic (e.g. LiH, CaC_2, Ba_3N_2) or essentially covalent (e.g. PbH_4, SnH_4 and BeC).

Some metal oxides, for example, alkali metal oxides (Na_2O, K_2O), react with water to give hydroxides (NaOH, KOH) which are very soluble in water, giving strong alkaline solutions. Other examples of metal hydroxides are $Zn(OH)_2$ and $Cd(OH)_2$.

Some transition metals form a variety of oxyhalides, ternary compounds containing a halogen element, oxygen and a metal. Examples of such compounds are $VOCl_3$, VO_2Cl, $CrOF_4$, MnO_3F and FeOCl.

Salts are ionic compounds formed by a reaction between metal hydroxides, such as sodium hydroxide (NaOH) and acids which may be either binary acids such as HCl, oxoacids such as H_2SO_4 (sulfuric acid), HNO_3 (nitric acid) and H_2CO_3, or organic acids such as acetic acid (CH_3COOH) or oxalic acid (HOOCCOOH). "Acid" salts are compounds such as sodium hydrogen carbonate $NaHCO_3$ or sodium hydrogen phosphate $NaH(PHO_3)$. "Basic" salts may be exemplified by magnesium hydroxide chloride Mg(OH)Cl; or $BiO(NO_3)$ and $BiO(OH){\cdot}BiO(NO_3)$ which are the products of hydrolysis of bismuth nitrite $Bi(NO_3)_3{\cdot}5H_2O$.

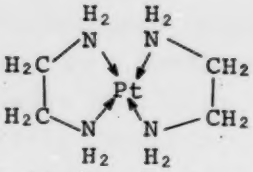
Transition metals such as manganese and chromium form, in their highest oxidation states, oxoanions such as manganate ion (MnO_4^{2-}), permanganate ion (MnO_4^-), chromate ion (CrO_4^{2-})

and dichromate ion ($Cr_2O_4^-$). Typical compounds containing such anions are potassium permanganate ($KMnO_4$) or lead chromate ($PbCrO_4$). Selenium and tellurium also form oxoacids, for example, H_6TeO_6 (telluric acid) and H_2SeO_4 (selenic acid). As regards the nomenclature of such polyatomic anions, the termination -ate indicates a higher oxidation state, e.g. arsenate AsO_4^{2-}, compared to an oxoanion with the central atom in a lower oxidation state, e.g. arsenite AsO_3^{3-}. The termination -ide is applied to binary compounds such as barium arsenide, Ba_3As_2.

3.3 Metal complexes

A metal complex is formed by the association of a metal atom or ion and another chemical species, called ligand, which may be either an anion or a polar molecule. Many complexes are rather stable structures and may remain unchanged in a sequence of chemical or physical reactions. All metals form complexes, but the formation of complexes is so widespread a phenomenon for transition metals that it provides a key for the understanding of their chemistry. Each metal can be assigned a principal valency which is the oxidation state. In addition, there is an auxiliary valency which represents the number of ligands associated with the central metal ion; this is equivalent to the coordination number of the metal. Most common are the coordination numbers 4 and 6, although others are known as well, ranging from 2 to 10.

Ligands can be unidentate, i.e. they provide only one electron pair. Examples are simple anions such as chloride or ammonia. Multidentate ligands contain more than one donor atom and it may be sterically possible to coordinate one metal atom at two or more positions in its coordination shell. A well-known example is ethylenediamine or 1,2-diaminoethane $NH_2 \cdot CH_2 \cdot CH_2 \cdot NH_2$ which contains two donor nitrogen atoms, and acts as a bidentate ligand. In some cases, such bi- or polydentate ligands form coordination compounds in the form of a heteroatomic ring, shown below. This process of ring formation is called chelation. Ligands such as ethylenediamine are often referred to as chelating agents.

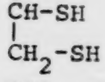


bis(ethylenediamine)platinum (II)

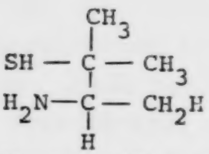
Chelates are usually very stable complexes and have therefore a great importance in analytical chemistry of metals and in the treatment of metal poisoning. Other multidentate ligands of great interest are nitrilotriacetic acid (NTA) which is tetradentate and ethylenediaminotetraacetic acid (EDTA) which is sexadentate.

A great variety of ligands exists. For example, they may have two basic groups as 1,10-phenanthroline, two acidic groups as carbonate ion, and one acidic and one basic group as is the case with α-amino acids, such as glycine. Corrin and porphin molecules are other examples of multidentate ligands which are of great biological importance, the corrin structure being the basic chelating arrangement in a cobalt complex, vitamin B_{12}.

The most useful chelating agents in the treatment of lead poisoning are EDTA and other aminopolycarboxylic acids. Arsenic is a "soft" Lewis acid (see section 5.3) which preferably binds ligands with sulfur donor atoms. This property has been used in developing a chelating ligand 2,3 dimercaptopropanol as an antidote to Lewisite, $Cl-CH=CH-AsCl_2$. BAL acts by forming a five-membered chelate ring with arsenic. BAL is effective in the treatment of mercury poisoning because mercury, like arsenic, shows a preference for ligands with sulfur donor atoms. D-isomer of penicillamine is the most effective chelating agent for binding copper. D-penicillamine can coordinate with a suitable metal through carbon, sulfur and nitrogen donors.



BAL



D-penicillamine

Further details on the chemistry and application of chelating agents can be found in a recent monograph by Bell (1977).

Another class of d-transition metal complexes of considerable toxicological interest are the complexes of π-acceptor (π acid) ligands. These are the complexes of a metal with various neutral molecules such as carbon monoxide, substituted phosphines, stibines, nitric oxide and pyridine, and include, for example, binary molecular compounds such as $Cr(CO)_6$ but also such complex ions as $[Fe(CN)_5CO]^3$ and $[Mo(CO)_5I]^-$. In many of these complexes the metal ion is in a low positive, zero or even negative formal oxidation state. The most important of these types of compounds are metal carbonyls which can be mononuclear, i.e. containing a single metal atom like $Fe(CO)_5$, or polynuclear like $Fe_3(CO)_{12}$, or heteronuclear like $MnRe(CO)_{10}$. Cyanide complexes such as $[Fe(CN)_6]^{4-}$ or $[Mo(CN)_8]^{3-}$ belong to the same group of metal compounds and are restricted to the d-block transition metals.

3.4 Organometallic compounds

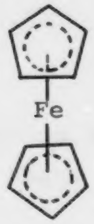
Organometallic compounds contain a carbon atom of an organic group bound to metal atoms. There are three main types of organometallic compounds:

1. Ionic compounds of electropositive metals. Examples of such compounds are $(C_6H_5)_3C^-Na^+$ or $(C_5H_5^-)_2\ Ca^{2+}$.
2. δ-bonded compounds in which the organic residue is bound to a metal atom by a normal 2-electron covalent bond. Examples of such compounds include $(CH_3)_3\ SnCl$ and CH_3HgCl.
3. Non-classically bonded compounds. One class of these compounds includes the alkyls of Li and Be that have bridging alkyl-groups. A second much larger class includes compounds of transition metals with alkenes, alkynes, benzene and other ring systems.

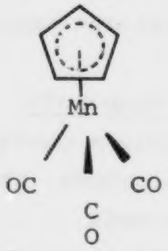
Of considerable toxicological interest are alkyl- or arylmercury compounds RHgX or R_2Hg. The RHgX type belongs to either ionic or δ-bonded compounds depending on X. When X can form a covalent bond with Hg, as for example Cl, Br, or OH, the compound is a covalent non-polar substance. When X is SO_4^{2-} or NO_3^-, the substance is ionic in character. Dialkyls and diaryls are non-polar, volatile, toxic, colorless liquids. Biological methylation of mercury or its compounds gives highly toxic $(CH_3)_2Hg$ or CH_3Hg^+. Methylcobalt oximes containing a $Co-CH_3$ bond can transfer CH_3 to Hg^{2+}.

Practically all the organometallic compounds of germanium, tin and lead are formed in the oxidation state +4. Of toxicological interest are only lead and tin compounds, particularly $(CH_3)_4Pb$ and $(C_2H_5)_4Pb$ and trialkyl and dialkyltins which have a variety of industrial and other uses.

Examples of the third category of organometallic compounds are η^5-cyclopentadienyls such as $(C_2H_5)_2Fe$. (η^5 indicates that 5 carbon atoms are bound to the metal, and is pronounced "pentahapto".) This type of compound is shown below.



ferrocene



η^5-cyclopentadienyl-Mn-tricarbonyl

Of potential toxicological importance are the cyclopentadienyl manganese tricarbonyl $(\eta^5 - C_2H_5)\ Mn\ (CO)_3$ and its methylated analog, MMT, which have been considered as a replacement for lead tetraethyl as an antiknock agent.

4. Solubility

The solubility of metal compounds in water and in lipids is of great toxicological importance because it is one of the major factors influencing the biological availability and absorption of metals. The solubility of metal compounds in water depends on the presence of other chemical species, particularly H^+ -ions (pH), and so it may be quite different depending on whether the solvent is "pure" water or a biological fluid. Biological fluids are slightly alkaline in mammals (pH 7.4) with the exception of the fluids in the gastrointestinal tract which are acid (pH 2-6) in the stomach and almost neutral (pH 6.8) in the intestines. In addition, biological fluids may also contain a variety of organic ligands which exert a further influence on the solubility. Experimental data on the solubility of metal compounds in biological fluids are very limited.

In spite of the mentioned differences, these are some simple rules governing the solubility of metal compounds in water which may be a useful indicator for the solubility of these compounds in biological fluids. A simple rule, used in chemistry, divides various substances into "soluble" and "insoluble". "Soluble" substances are those which have a solubility in water >1 g/100 ml; "insoluble" are those which have a solubility < 0.1 g/100 ml. This distinction may not be meaningful if a substance is highly toxic. Within each group of the periodic system the solubility of metal compounds generally decreases with increasing atomic numbers.

Mainly soluble metal compounds are nitrates, acetates and all chlorides, bromides and iodides except those of silver, mercury (I) and lead. All sulfates, except those of barium, strontium and lead are also soluble. All salts of sodium, potassium and ammonium are soluble except sodium antimonate, potassium hexachloroplatinate and potassium cobaltonitrite.

Mainly insoluble are all hydroxides (except those of alkali metals, ammonium and barium), all normal carbonates and phosphates (except those of alkali metals and ammonium), and sulfides (except those of alkali metals, ammonium and the alkaline earth metals).

In addition to the factors mentioned above, such as pH, the presence of other ions, etc., solubility may depend on the oxidation state of a metal, and on the rate of oxidation/reduction conversions.

The solubility of sparingly soluble substances will also depend on their particle size. Finely divided material is usually more soluble.

5. Properties of metal ions

5.1 Formation of metal ions

Metals ions are formed by the removal of one or more outer electrons from the neutral atom. The energy required for the ion formation depends on the environment in which this process takes place. The formation of ions in the gas phase requires a considerable amount of energy. The same process requires much less energy if it takes place in water because a part of the ionization energy is provided by the energy of hydration, i.e. the energy that is gained when a positively charged metal ion binds dipolar water molecules. The number of water molecules that are bound directly to the metal ion (first hydration sphere) depends on the size and the charge of the metal ion, and varies from 4 for Li^+ to about 10 for Ra^{2+}. Because there is further polarization of water molecules contained in the first hydration sphere, additional water molecules will be attracted to form a second hydration sphere. This association can, of course, continue but its extent decreases rapidly with distance from the ion. The size of the hydration sphere will obviously depend on the polarizing power of the ion which in turn depends on the charge/radius ratio. The hydrated ion is a dynamic system in which water molecules in the hydration sphere rapidly exchange with those in the bulk phase of the solution.

5.2 Redox potentials

The removal of electrons from solid metal is an oxidation process. The reverse process is called reduction. Oxidation and reduction processes are always coupled, i.e. when one substance is oxidized (reducing agent), another must be reduced (oxidizing agent). The oxidizing or reducing power of an oxidation-reduction system is measured in terms of standard electrode potentials. If the standard electrode potential of a metal is large and negative, the metal is a powerful reducing agent because it loses electrons easily. Standard electrode potentials, or redox potentials, depend on the concentration of metal ions, on the temperature, and on the presence of other ligands that can displace water from a hydrated ion. Oxidation-reduction reactions are of fundamental importance in biochemistry, and transition metals which can easily change their oxidation state play a very important role.

5.3 Metal ions as Lewis acids

A useful definition of acids and bases is that of G.N. Lewis who defined an acid as an electron pair acceptor and a base as an electron pair donor. Thus all the positively charged metal ions can be classified as Lewis acids or electron acceptors. In the same way water molecules which are formally electron donors can be described as Lewis bases. In this terminology the formation of a hydrated metal ion is in fact the formation of a complex. This can be indicated by the following equations:

Metal ion + water → hydrated metal ion
(Lewis acid + Lewis base → complex)
Hydrated ion + ligand → complex and water

The last equation indicates that water in the hydration shell can be replaced by another ligand (another Lewis base) to give a different complex. The chemistry of metal complexes is very important not only in analytical chemistry but also for the understanding of metal biochemistry and toxicology. Lewis acids and bases can be classified as "hard" and "soft". A "hard" metal ion or Lewis acid is one which retains its valence electrons very strongly and is not readily polarized. Ions of small size and high charge are "hard". A "soft" ion is relatively large, does not retain its valence electrons firmly and is easily polarized. Examples of hard ions are L^+ and Mg^{2+} as well as bases with donor atoms such as oxygen, nitrogen (e.g. amines, ammonia, water) or fluorine. "Soft" metals include copper (I), silver (I), gold (I), thallium (I), palladium (II) and platinum (II). Easily polarized ligands containing phosphorus, arsenic or sulfur donor atoms are "soft" bases.

As a rule the formation of stable complexes results from interaction of hard acids with hard bases, or soft acids with soft bases. The bonding in hard-hard complexes is largely electrostatic whereas in soft-soft complexes it is more covalent.

5.4 Hydrolysis

Hydrolysis is a reaction that may occur between the metal ion and a water molecule in the coordination (solvation) sphere, in which a proton (hydrogen ion) is released and the solution becomes acidic. This can be described by the equation

$$\left[M\ (OH_2)x\right]^{n+} \rightleftharpoons \left[M(OH_2)x-1\right]^{(n-1)+} \quad + \quad H^+ \quad \text{(a)}$$

Hydrolysis may proceed in several stages until the last coordinated water molecule is removed. The process of hydrolysis may be interrupted if at one stage an insoluble compound is produced. Hydrolysis occurs most readily with metal ions which strongly polarize the coordinated water molecules. The polarizing power increases with the increasing charge and decreasing size of the ions. For example, in the case of alkaline earths, the hydrolysis is confined to Be^{2+} which has a small radius compared to other members of the group.

5.5 Main group metals

The main group metals are those belonging to the periodic system groups Ia (alkali metals), IIa (beryllium and alkaline earths), IIb (zinc, cadmium and mercury), IIIb (aluminum, gallium, indium and thallium), IVb (germanium, tin and lead), Vb (antimony and bismuth). Arsenic (group Vb), selenium and tellurium (group VIb) are metal-like elements that also belong to the main group elements.

The salts of the alkali metals are generally soluble and produce unipositive aquo ions with a probable coordination number 4 for Li, Na and K and a probable coordination number 6 for Cs. Lithium salts are frequently less soluble in water than the corresponding salts of other alkali metals. The hydrated cations of the alkali metals show little tendency to hydrolyze, or to form complex ions. Potassium and sodium have important biological functions (e.g. osmo-regulation, acid-base balance in the organism, transmission of nerve impulses) and lithium is of toxicological interest. By losing its outer s-electron, lithium +1 ion becomes spherically symmetrical and non-polarizable. The similarity in chemistry of Li^+ and Mg^{2+} can be explained by their similarity in charge/radius ratios.

The salts of strong acids of alkaline earths are soluble, with the exception of the sulfates of Ca, Sr and Ba. Salts of weak acids are considerably less soluble. All the hydroxides with the exception of Be-hydroxide are strong bases but much less soluble than the corresponding alkali-metal compounds. Be-hydroxide is amphoteric. All cations of this group are hard Lewis acids and bind best anionic oxygen donor ligands such as polyphosphates and carboxylates. Of the six elements only magnesium and calcium have a definite biological role (e.g. enzyme activation, muscular contraction, skeleton formation); all others are toxic to varying degrees. Beryllium toxicity might be due to its capacity for forming strong covalent bonds, thus displacing other divalent cations. For example, even in concentrations as low as 10^{-6} mol dm $^{-3}$, it inhibits *in vitro* alkaline phosphatase which is magnesium-activated. Barium toxicity could be due to its interference with the calcium function.

There is a great similarity in properties between zinc and cadmium, and the Zn^{2+} and Cd^{2+} ions are similar in many respects to Mg^{2+}, particularly as regards the solubility of halides and oxo-acid salts. In contrast to Mg^{2+}, zinc and cadmium ions are rather strong Lewis acids. The salts of Hg^{2+} with strong oxo-acids (H_2SO_4, HNO_3) are soluble but at the same time hydrolysis will occur because mercury (II) hydroxide is a weak base. Hg^{2+} cation forms coordination compounds with a coordination number 4 but has a pronounced tendency towards complex formation with a coordination number 2 as well. If elemental mercury is present, mercury (II) ion will disproportionate to form mercury (I) ion, $^{+}Hg - Hg^{+}$, with a strong metal-metal bond.

Because metals of group IIb have a filled set of d-orbitals interposed between the core and the valence electrons, and because these d-orbitals provide only a weak shield for the increased nuclear charge, the ionization energies of Zn, Cd and Hg are higher than those of Ca, Sr and Ba. The result is that the complexes of Zn^{2+}, Cd^{2+} and Hg^{2+} have a considerable covalent character due to the strong attraction these cations exercise on electrons. For the same reason, the sulfides of Zn, Cd and Hg are insoluble. The increase in covalency with increasing nuclear charge is also demonstrated by the stability of metal alkyls of mercury, a fact of considerable toxicological and ecological importance. Zinc has an important biochemical function as potentiator of many enzymes. In contrast, cadmium and mercury have no known biological role. Cadmium toxicity may be related to its similarity to zinc. In the mammalian kidney, cadmium has been found in a ternary Cd-Zn protein complex, metallothionein, which has up to 10% cadmium and a very high content of cysteine residues. The toxic effects of mercury appear to be related to its ability to bind thiol groups, which is a consequence of the affinity of both cadmium and mercury for sulfur ligands.

Boron, the first element of group IIIb, is a non-metal. Aluminum, the next member of the group, has a larger atomic radius and consequently a lower ionization potential. Therefore, it behaves like a metal and so do other members of the group, i.e. Ga, In and Tl. The salts of strong oxo-acids are soluble, but those of weak acids undergo extensive hydrolysis. All the members of this group form compounds in the oxidation state +3, but because of the very low first ionization potential, in some cases univalent ions are also known in aqueous solutions (e.g. In^+ and Tl^+). In fact Tl^+ is usually more stable than Tl^{+3}. Chemically, Tl^+ is similar to K^+ and Ag^+. Complex formation is confined almost entirely to ligands having oxygen donor atoms and to the halide ions.

In group IVb, only lead has strongly pronounced metallic properties. When the cations are in the oxidation state +4, the high charge and the small size will provide a strong polarizing power leading to compounds of covalent character. The stability of compounds in +4 oxidation state decreases down the group. When in lower oxidation state +2, tin and lead form typical ionic compounds. The covalent character of the +4 oxidation state is reflected in the wide range of organometallic compounds formed by germanium, tin and lead.

The elements of group Vb and group VIb show an increasingly non-metallic character. In group Vb, antimony and bismuth are considered to be metals, but they show many non-metallic properties. In group VIb only polonium is a metal.

None of these metals has a defined biochemical role. Gallium and indium are moderately toxic, but thallium is a very toxic element which seems to interfere with sodium-potassium metabolism. Germanium has no known biological role; the role of tin is uncertain and lead is very toxic.

5.6 Transition metals

The usual definition of the transition elements is that they have, as elements, partly filled d or f shells. It is, however, more appropriate to include also those elements which have partly filled d or f shells in any of the oxidation states in which they form compounds. This broader definition includes among transition elements also Cu, Ag and Au. There are about 56 transition elements of the d- and f-block. All of them have some common properties:

1) they are all metals;
2) with a few exceptions, they exhibit variable oxidation rates;
3) because of partly filled shells, they form at least some paramagnetic compounds;
4) their ions and compounds are colored in one or all oxidation states.

Properties 2) and 3) are of great biological importance and make some of them particularly suitable for biological catalysis and for electron transport function.

The transition elements are further subdivided into three main groups:

a) the main transition elements or d-block elements
b) the lanthanide elements, and
c) the actinide elements.

The chemistry of transition elements is extremely complex and the present discussion will be limited to the d-block elements. Among them, of particular biological and toxicological interest are some members of the first row, i.e. Ti, V, Cr, Mn, Fe, Co, Ni and Cu. In the second row Mo is certainly of greatest toxicological interest, and to some extent Ru, Rh, and Pd. In the third row attention should be paid to the triad Os, Ir and Pt.

The chemistry of the d-block cations is dominated by their ability to act as Lewis acids forming complexes with Lewis bases. With ligands of biological interest having nitrogen, oxygen and sulfur donor atoms, the most stable complexes are formed in the +2 and +3 oxidation states. An important feature of such compounds is that under very similar conditions one can obtain complexes that differ only with respect to the oxidation state of the metal. Examples are $[Co^{II}(NH_3)_6]^{2+}$ and $[Co^{III}(NH_3)_6]^{3+}$ which can both be prepared at room temperature. The Co^{II} complex can be easily oxidized by oxygen to the Co^{III} complex. The process can be easily reversed by a suitable reducing agent. This ability to move easily between the two adjacent oxidation states is particularly valuable in the biological transport of electrons.

The simplest theory that has been developed to explain the behavior of transition metal ions and complexes is the crystal field theory. This theory makes the assumption that all ligands, including water, can be treated as point negative charges, subject only to the laws of electrostatics.

The reactions of the transition-metal complexes include ligand substitution, redox processes and reactions of coordinated ligands. These and other topics of interest for the understanding of the biochemistry and toxicology of some transition metals are discussed in detail in a monograph by Hughes (1972).

7. Biological molecules containing metals

Many metals have very important biological functions which they achieve in most cases in the form of complexes with other biological molecules. An excellent treatment of this aspect of biochemistry may be found in Hughes' monograph (1972).

7.1 Metalloporphyrins

The metalloporphyrins include two extremely important ones: the chlorophyll molecule and the heme group. The ability of the chlorophyll molecule to absorb light is related to the conjugated polyene structure of the porphyrin ring. Magnesium ions which are coordinated to the nitrogen atoms of the four pyrrole rings have at least two functions: to provide the necessary structural rigidity and to increase the rate of conversion of the singlet excited state resulting from photon absorption into the triplet state which enables the transfer of the excitation energy into the redox chain.

The two main functions of iron-containing biological complexes are the transport of oxygen and mediation in electron transfer chains. The heme group is in all cases associated with a protein molecule as in hemoglobin, myoglobin, cytochromes and enzymes such as catalase and peroxidase. Cytochromes serve as electron carriers and the heme-containing enzymes, catalase and peroxidase, catalyze the decomposition of hydrogen peroxide.

7.2 Non-heme iron proteins

Non-heme iron proteins, such as rubredoxins, ferredoxins and high potential iron proteins (Hi PIPs) contain strongly bound functional iron atoms attached to sulfur, but they do not contain porphyrins. All of them participate in electron transfer processes.

The third group of iron-containing biological structures are ferritin and hemosiderin. Both serve to store iron in a protein structure. Transferrin binds ferric iron and transports it from ferritin to red cells. In microorganisms, iron is transported by ferrichromes and ferrioxyamines, structures containing cyclic or acyclic polypeptide chains.

7.3 Cobalt-containing biological molecules

The best known biological molecules containing cobalt are the vitamin B_{12} coenzymes (cobalamines). Cobalamines consist of a cobalt atom, a microcyclic ligand corrin, and a complex organic part containing a phosphate group, a sugar and an organic base also coordinated to the cobalt atom. Methylcobalamine is involved in the methane-producing bacteria and transfers the CH_3 group to Hg^{II}, Tl^{III}, Pt^{II} and Au^{I}.

7.4 Metalloenzymes and metal-activated enzymes

Some enzymes incorporate one or more metal atoms in their normal structure. They are called metalloenzymes of which at least 50 have been identified. About 20 of them incorporate zinc. The best known members are carbonic anhydrase and carboxypeptidase. The structure of carboxypeptidase is partly known. The zinc ion is bound in a distorted tetrahydral configuration , with two histidine-nitrogen atoms, one glutamate carboxyl oxygen atom and a water molecule as ligands.

There are about 15 copper-containing metalloenzymes. Their structure is not well understood. Examples are ascorbic acid oxidase and various tyrosinases. In many lower animals (crabs, snails) the oxygen carrying molecule is a copper-containing protein hemocyanin which, however, does not contain any heme groups.

Metalloenzymes containing molybdenum and iron (nitrogenases) play an important role in nitrogen fixation.

Metal-ions can be bound to proteins in a reversible way. This is the case with metal-activated enzymes. Such systems are much less amenable to study than metalloenzymes, because they cannot be isolated with metal ion in place. Most enzymes associated with phosphate group transfer or hydrolysis appear to be activated by Mg^{2+}.

REFERENCES (General)

Bell, C.F. (1977). "Principles and Applications of Metal Chelation." Clarendon Press, Oxford.

Brakhanova, I.T. (1975). "Environmental Hazards of Metals." Consultants Bureau, New York.

Cotton, F.A. and Wilkinson, G. (1972). "Advanced Inorganic Chemistry." 3rd edition. Interscience Publishers, New York.

Cotton, F.A. and Wilkinson, G. (1976). "Basic Inorganic Chemistry." John Wiley & Sons, New York.

Hughes, M.N. (1972). "The Inorganic Chemistry of Biological Processes." John Wiley & Sons, New York.

Parr, G. (1973). "Ions in Solution (3), Inorganic Properties." Clarendon Press, Oxford.

Pauling, L. (1970). "General Chemistry." W.H. Freeman & Co., San Francisco.

Phipps, D.A. (1976). "Metals and Metabolism." Clarendon Press, Oxford.

Table 1. Periodic table of the elements

Period	Group Ia	Group IIa	Group IIIa	Group IVa	Group Va	Group VIa	Group VIIa	Group VIII			Group Ib	Group IIb	Group IIIb	Group IVb	Group Vb	Group VIb	Group VIIb	Group O
1 1s	1 **H**																1 **H**	2 **He**
2 2s2p	3 **Li**	4 **Be**											5 **B**	6 **C**	7 **N**	8 **O**	9 **F**	10 **Ne**
3 3s3p	11 **Na**	12 **Mg**											13 **Al**	14 **Si**	15 **P**	16 **S**	17 **Cl**	18 **Ar**
4 4s3d 4p	19 **K**	20 **Ca**	21 **Sc**	22 **Ti**	23 **V**	24 **Cr**	25 **Mn**	26 **Fe**	27 **Co**	28 **Ni**	29 **Cu**	30 **Zn**	31 **Ga**	32 **Ge**	33 **As**	34 **Se**	35 **Br**	36 **Kr**
5 5s4d 5p	37 **Rb** s	38 **Sr**	39 **Y**	40 **Zr**	41 **Nb**	42 **Mo**	43 **Tc** d	44 **Ru**	45 **Rh**	46 **Pd**	47 **Ag**	48 **Cd**	49 **In** p	50 **Sn**	51 **Sb**	52 **Te**	53 **I**	54 **Xe**
6 6s (4f) 5d 6p	55 **Cs**	56 **Ba**	57• **La**	72 **Hf**	73 **Ta**	74 **W**	75 **Re**	76 **Os**	77 **Ir**	78 **Pt**	79 **Au**	80 **Hg**	81 **Tl**	82 **Pb**	83 **Bi**	84 **Po**	85 **At**	86 **Rn**
7 7s (5f) 6d	87 **Fr**	88 **Ra**	89•• **Ac**															

•Lanthanide series 4f	58 **Ce**	59 **Pr**	60 **Nd**	61 **Pm**	62 **Sm**	63 **Eu**	64 **Gd**	65 **Tb**	66 **Dy**	67 **Ho**	68 **Er**	69 **Tm**	70 **Yb**	71 **Lu**
••Actinide series 5f	90 **Th**	91 **Pa**	92 **U**	93 **Np**	94 **Pu**	95 **Am** f	96 **Cm**	97 **Bk**	98 **Cf**	99 **Es**	100 **Fm**	101 **Md**	102 **No**	103 **Lr**

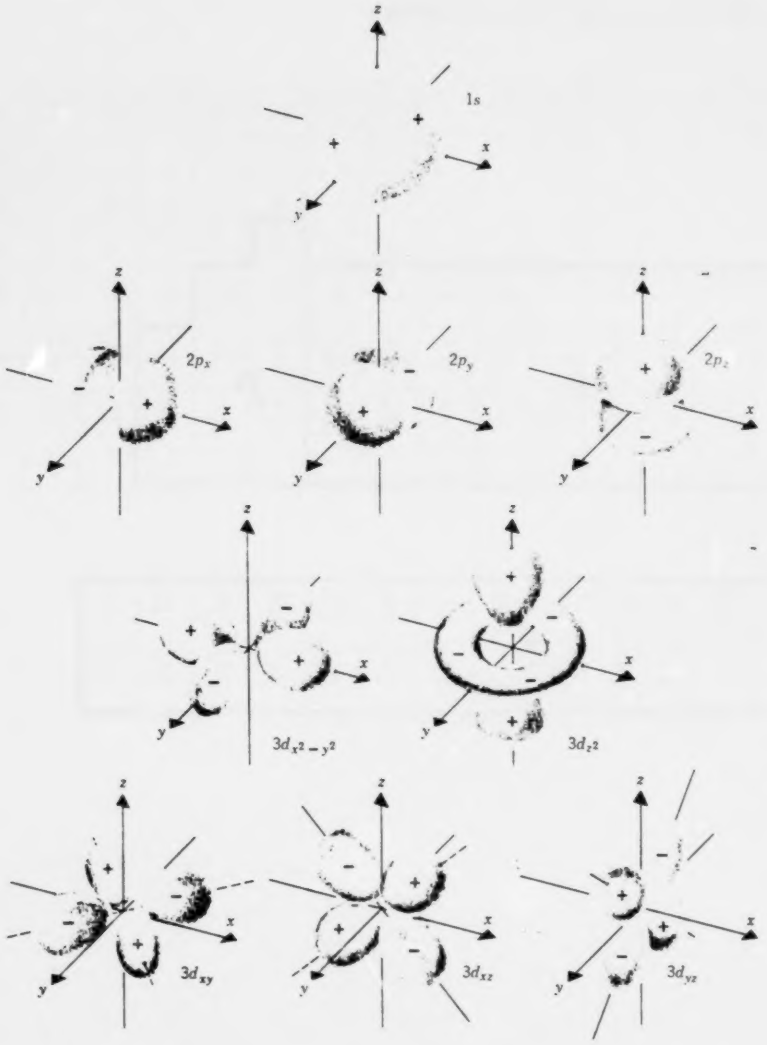

1s
2p_x
2p_y
2p_z
3d_{x^2 - y^2}
3d_{z^2}
3d_{xy}
3d_{xz}
3d_{yz}
x
y
z
+
−

Figure 1. Shapes of atomic orbitals

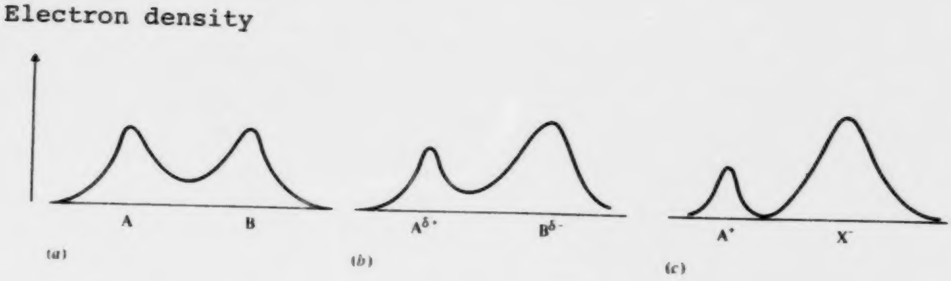


Figure 2. Electron distributions in (a) homopolar covalent bond (b) heteropolar covalent bond with partial ionic character (c) ionic bond

SAMPLING AND ANALYTICAL METHODS

Magnus Piscator and Velimir Vouk

Dose estimation involves several analytical steps, and the final result will of course depend on how well both the sampling and the analysis have been performed. In many well designed studies from the epidemiological point of view where great care has been taken to obtain good response data, the analytical methods used have not been checked for accuracy. The results of these studies could therefore not be used for dose-response estimation. There have also been many instances in which investigators have combined data obtained in epidemiological studies with data on doses of metals in different media recorded in previously published studies. An attempt to construct dose-response curves in that way may often be disastrous. For example, in a recent paper cancer mortalities in different countries were related to assumed dietary intakes of certain metals, and the daily intake of cadmium in Sweden and USA was assumed to be about twice as high as that in Japan (Schrauzer et al., 1977). The data on intake had been published more than 10 years ago. Recent data (see Friberg et al., 1977) obtained by modern analytical techniques were completely neglected. The opposite may also happen, i.e. laboratories with good analytical experience, capable of making accurate measurements on metals in different media, may start conducting epidemiological studies which they are not capable of designing properly. Results concerning dose-response hereby obtained may be quite misleading.

This chapter provides some general comments about sampling and analysis which are intended primarily to aid industrial physicians, epidemiologists and toxicologists to interpret analytical data at their disposal. It is not intended to give details on the advantages and disadvantages of specific sampling techniques and analytical methods. Such information is contained in the chapters of Volume II of this series, metal by metal.

Preceding page blank

Much information on sampling and analysis can be found in the two volumes "Accuracy in Trace Analysis, Sampling, Sample Handling, Analysis" edited by LaFleur (1976) and in the monograph by Winefordner (1976).

1. Sampling

1.1 General considerations

The exposure dose may be estimated from measurement of metal concentrations in environmental media (air, water, food) as a function of time. Determination of exposure dose is of course of great importance since it makes it possible to observe trends in pollution. Of still greater importance is the assessment of the dose in tissues or organs. This can be done either by applying appropriate metabolic models to the exposure dose measured, or by measuring concentrations in the tissue or organ of interest directly. Another possibility is to estimate tissue or organ dose from the measurement of the concentration of the metal in index media, e.g. blood, urine, hair etc. Here again we need metabolic models.

Only in a few instances can exposure dose be directly related to absorbed dose. For example, it is known that methylmercury is absorbed nearly 100% from the intestine. By measuring methylmercury in fish meat and at the same time knowing daily intake of fish, it is thus possible to calculate the dose. For many other metal compounds, however, accurate data on the rate of absorption from the lungs or the intestine are lacking and internal dose measurements must be made.

1.2 Air

When evaluating exposure to metals in air it is important to know not only the actual concentrations but also the particle size distribution and the solubility of the metal compounds. This is especially useful when discussing occupational exposure, where both highly soluble and highly insoluble particles of different sizes may occur at the same time.

1.2.1 Ambient air

The concentrations of metals in ambient air are generally low, and intake through air is usually small in relation to intake via food. Exceptions are areas in the vicinity of plants emitting large amounts of metals, e.g. smelters, and heavily trafficked areas, where the intake of lead through air may exceed the intake with food and water. In some countries a continuous measurement of metals in both rural and urban air has been carried out for many years. Sampling has been performed at stationary sites often located in such a way that the exposure of people living in these areas could not be estimated adequately. The great significance of these continuous measurements is that they provide information about trends in general air pollution. Moreover, combined with data on the fall-out of metals, they may be used for predicting the long-term accumulation of metals in soil and in certain food chains.

There is an abundance of data from many countries on the levels of lead in air in areas with heavy traffic as well as in rural areas. Such measurements have often been made with stationary samplers. Attempts to correlate the levels of lead in blood in persons in these areas with the lead levels in air measured there, have generally shown no or very weak correlations. These findings stem from the fact that the subjects under investigation have a great mobility and are exposed to varying concentrations of lead during the day, whereas a stationary sampler only determines lead at a certain point, generally not in the breathing zone. The varying intake of lead via food also makes it difficult to define the relationships between lead in ambient air and lead in blood. There is one study (Azar et al. (1973) where personal samplers were used for continuous recording of airborne lead, and where blood lead was repeatedly determined in several exposed groups. However, as the authors themselves pointed out, the lack of adequate data on dietary intake made it difficult to determine the quantitative relationship between airborne exposure and blood levels of lead. Recommendations on sampling systems for ambient air are found in reports by Katz (1969) and WHO (1977). Statistical problems concerning air pollution measurements have been treated by EPA (1976).

Long-term deposition of metals through fall-out is also of interest. The fall-out is generally collected in a deposit gauge of standardized size which makes it easy to compare data from different countries. In most cases the sample is collected for one month and the results are expressed as mass per m^2 and month. The deposit gauge must be protected from contamination by birds and other animals. During its long period of use, the method has proved to be of great value for monitoring emissions from plants.

Another valuable means of arriving at the long-term deposition of airborne metals is to collect and analyze certain types of mosses growing on rocks or other supports, and taking their nutrients mainly from the air. These mosses have a great capacity for absorbing metals. In the Scandinavian countries (Rühling and Tyler, 1973) and the U.K. (Goodman and Roberts, 1971) this approach has provided valuable information.

1.2.2 Industrial air

Occupational exposure takes place mainly by inhalation and good sampling techniques and accurate analysis are thus essential for evaluating the exposure dose in such environments. The concentrations of metals in air are generally much higher than in ambient air, which makes it easy to collect sufficiently large amounts. The analysis will also be easier, since the interference from other elements is much less than in the ambient air. One great problem still lies in obtaining information on the amount of metal in the breathing zone of the exposed workers.

Previously, stationary samplers were generally used and the results have been of value when looking at trends in the working environment. Such results cannot, however, be used for estimating individual exposure, except in the case of workers who have a great mobility in the work shops, e.g. foremen. During recent years personal samplers have been more and more in use, and do of course give better estimates of the amount of a metal actually inhaled. Several points must be taken into account when interpreting data obtained by personal sampling.

1. Dust on the worker and his clothing may contaminate the filter, resulting in too high estimates.

2. The personal sampling data for some metals such as cadmium may give a very inaccurate picture of the total exposure, if personal habits, especially smoking, which might increase the exposure substantially are not considered (Piscator et al., 1976). Adamsson et al. (to be published) found that workers exposed to cadmium oxide dust (<10 $\mu g/m^3$) had a fecal elimination of cadmium in some cases of 1-3 mg, only part of which could be attributed to mucociliary clearance, the main part probably coming from oral contacts with contaminated hands or cigarettes.

3. The concentrations of a metal, as well as other substances, measured in air at a certain point have a log-normal distribution, for which reason the distribution should be summarized in terms of the geometric mean and standard deviation. For estimating individual exposure and compliance or noncompliance with occupational health standards the arithmetic mean should be used, since that reflects the actual inhaled amount. Confidence limits for the arithmetic mean, however, must be calculated on the basis of log-normal distribution. For details see Coenen (1966), Sherwood (1971), Leidel and Busch (1975), and Ulfvarson (1977).

1.3 Water

In areas with running water, cool tap water is generally used for preparing food, but hot water may also be used, i.e. for baby food. It might thus be of importance to determine metals in both the cool tap water and the hot tap water. The amount of tap water imbibed directly, versus that used for preparing hot beverages and for cooking food must be carefully estimated, since the biological availability of metals may be quite different under the two circumstances. The intake of drinking water may vary considerably depending on individual and local habits and on climate (ICRP, 1975; Zoeteman and Brinkmann, 1976). Since water pipes may contain materials that can release certain toxic metals in larger amounts, concentrations of metals should be examined in the first portion of water tapped after the faucet has been turned off for some time, and also in the water after it has been running for some time. Especially lead and cadmium may accumulate during longer periods when water is not tapped. Estimates of community exposure based on data from the analysis of metals in water from waterworks may lead to erroneous conclusions. Here and otherwise it should be kept in mind that people may often drink water from different supplies during the day (work-place, travel etc.). Furthermore, factors like hardness and pH should be determined, since they might be of importance for exposure evaluations. Since the concentrations of most of the metals of interest are low, extremely good care must be taken in cleaning the vessels for collecting of samples. The glass or plastic bottles must be acid-cleaned and, before use, checked for leaching of the metals under study. For more details see EPA (1972), APHA (1976), and Maienthal and Becker (1976).

1.4 Food

At least three methods are available at present for estimating intake of metals via food products. One method is to collect samples of single foodstuffs, analyze them for the metal under consideration, and then estimate the amount of metal that could be ingested by people with different food habits, given the available food statistics. A second method is to collect certain classes of foods, i.e. vegetables, dairy products, fish, and meat products in the amounts that are actually consumed, analyze each class and make estimates from that. Prior to analysis, each food should be treated as it would be normally, e.g. cooked. The so-called market basket studies in the USA have been based on this method (for details see Mahaffey et al., 1975). A third method is the duplicate samples method. During a certain period, the people under study put meals identical to the ones they have eaten into a vessel. The deposited meals can be homogenized and analyzed, providing a total intake figure. The first of the three mentioned methods is the simplest and has been recommended by FAO/WHO (1977). The disadvantage is that only raw products are examined. The second method requires larger resources, but gives more accurate estimates. The third method gives good information on individual intakes, but can only be performed on a limited number of subjects. For details on sampling and analysis of foods, see Crosby (1977).

Another method which can be used on the general population is to determine the fecal elimination of the metal in question. Results of studies in Japan, USA, and Sweden show that the estimates of dietary intakes of a metal like cadmium with low gastrointestinal absorption thus obtained are of the same magnitude as those obtained by the more laborious methods described above (see Friberg et al., 1977). Occupationally exposed persons must be carefully excluded from such studies since they might eliminate in feces metals cleared from the lungs. The same care must obviously be taken as for water samples in preparing the collecting vessels.

1.5 Body fluids and tissues

Metal concentrations in blood and urine are generally low and great care must be taken to avoid contamination from sampling equipment. Acid-cleaned test tubes must be used for collection of blood samples. Preventive measures must be taken lest some materials, like the rubber in stoppers, release metals into the sample. Blood is usually taken from the cubital veins and stainless steel needles suitable for collection of blood for serum-iron determination may be used to extract samples for most metals. Special care must be taken when chromium or nickel are to be analyzed, since these metals may be present in steel. In the industrial environment caution must also be exercised that the metal under investigation is not transferred from the skin to the needle. A special problem is the collection of blood from small children when lead poisoning is suspected. It is in such cases often impossible to obtain samples from the cubital vein and instead, capillary blood is drawn from the finger tips. There is a risk for severe contamination from the skin when sampling that way.

Urine should be collected in acid-cleaned plastic bottles and if exposed workers are being examined, urine specimens should be collected at home to avoid any contamination with dust from the working environment. Morning samples are superior to random samples as far as standardization of the procedure is concerned. Determination of specific gravity or creatinine in urine is useful when correcting for the great variations in dilution of urine.

Data on metal concentrations in organs are generally obtained from autopsy samples. There are very few instances in which it has been possible to study the metal concentration in organs from living people.

Generally tissue sections can be obtained with stainless steel knives. Exceptions are again nickel or chromium for which special glass knives or other suitable instruments should be used. When sampling some organs, e.g. kidney, it is important to cut from the same part every time. Many metals are unevenly distributed in the different layers of the organ.

Blood, urine and organ samples can be stored for a long time before analysis, but if the sample volume is small in relation to that of the container, the wet weight might change due to the loss of water. For more details, see Mertz (1975), Anand et al. (1975), Maienthal and Becker (1976), and a recent report by CEC/WHO/EPA (1977).

2. Analytical methods

2.1 General aspects

Assuming that correct sampling has been performed, the next step is quantitative analysis. Regardless of which analytical method is used, some points must always be carefully considered. These are accuracy, precision and detection limit. Accuracy can be defined as a systematic deviation from the "true value". Precision is generally expressed in terms of the coefficient of variation, also called RSD (relative standard deviation), arrived at by analyzing the same sample a number of times over a certain period. Precision should be measured under conditions that correspond to the ones prevailing in the regular analytical work. It should be noted that both accuracy and precision depend on the concentration of the metal to be analyzed. Limit of detection is the minimum amount or concentration that the analytical method is able to distinguish from a zero concentration. Limit of detection is of less importance from the toxicological point of view than the other two measures of analytical performance. It may often be sufficient to know that a concentration is below a certain level, provided that accuracy and precision are good.

Analysis involves many steps, from the moment the sample arrives at the laboratory until the final reading is obtained. The main ones are homogenization of samples, ashing, extraction, separation, and chemical or physical analysis (see e.g. Crosby, 1977). The media to be examined may exhibit large differences in matrix composition and metal content. There are no standard procedures applicable to all types of media. For example, the determination of cadmium in renal cortex is a relatively simple procedure, whereas that in blood and urine is highly complicated due to the low concentration of cadmium and the complex matrix composition, with many interfering substances.

The information in the following sections relies to a large extent on the monographs by Winefordner (1976), and Pecsoc et al. (1976).

2.2 Current methods for analysis of metals

Some twenty years ago, colorimetry, emission spectrography, and polarography were the dominating methods for the quantitative determination of metals. Atomic absorption spectrometry was then in the introductory stages for trace metal analysis. By the middle of the 1960's, atomic absorption equipment had become commercially available and this method was being employed routinely for quantitative determination of metals by more and more laboratories. Tremendous progress in the refinement of the atomic absorption method has taken place during the late 1960's and the 1970's. At present, it is the most commonly used method. However, many of the earlier methods are still employed and new methods are being developed which may become strong competitors to atomic absorption methods in the future. In the following sections, some of the most common methods will be discussed mainly with regard to basic principles, applicability and limitations. Details pertaining to the use of a given method for a specific metal are taken up in Volume II of this series.

2.2.1 Spectroscopic methods

2.2.1.1 Colorimetry and absorption spectrophotometry

Colorimetry, the first modern method to be developed for metal analysis, is based on the formation of colored complexes between metals and organic compounds, whose absorbance can be measured in a colorimeter or spectrometer, after extraction into a suitable solvent. Using current terminology colorimetry may also be classified as electronic absorption spectrometry. The basic principle is simple, i.e. a specific complex is formed which absorbs light in a given spectral region. Only a few metals form specific complexes that can be directly extracted and analyzed, as in e.g. the classic procedure for determination of serum-iron with bathophenantroline. For most metals, several separation steps must be taken before the complex can be extracted. A typical example is the determination of cadmium with dithizone where cadmium dithizonate must be separated from e.g. zinc and lead dithizonates.

Colorimetric methods still have many applications since the more modern methods are not suitable, or in some cases, have not yet been worked out, for some of the metals. NIOSH (1974) recommended that antimony in urine should be determined by a colorimetric method, the Rhodamine B method. SCOPE (1975) recommended a method involving methylene blue for selenium in air and a dithiocarbamate method for arsenic in air. Metals of greatest interest at present, e.g. cadmium, lead, and mercury, can all be determined with colorimetric methods, especially using dithizone, but atomic absorption has been shown to be superior. The limitations of the colorimetric methods are mainly that much work has to be performed in order to isolate the specific complexes. This work is expensive, time-consuming and entails a high risk of contamination, as well as occupational hazards to the analysts.

2.2.1.2 Emission spectrometry

Atomic emission spectrometry is based on the excitation of metal atoms by flames or by electric discharges. The excited atoms then lose their acquired energy by emitting photons. This emission can be recorded on a spectrometer or spectrograph which has a monochromator that separates the emitted radiation according to wave-lengths (i.e. the energy of photons). A spectrum is obtained and a very wide range of metals can be more or less quantitatively determined. Atomic emission spectrometry is especially useful for multi-element analysis, e.g. for soil analysis, geological surveys etc. It was used by Tipton and coworkers (1963) in extensive investigations on the metal content in different organs of the human body, but accurate data could not always be obtained. Atomic emission spectroscopy is also used in the metal industry for the quality control of products. In many industries spectroscopic equipment is also used for monitoring the exposure of workers to such metals as lead and cadmium. Emission spectrometric methods are not suited to the determination of easily volatile substances like selenium, arsenic or mercury.

The standards and the samples to be analyzed must have matrices of the same composition, since the intensity of the emissions can be affected by the matrix. The electrical discharge sources may vary. For example, the DC arc source does not give a better reproducibility than about 20%. The DC arc enables, however, a low detection limit. Other spark sources may give a better reproducibility, but a higher detection limit. For the best type of equipment, the cost is very high.

2.2.1.3 Atomic absorption spectrometry

The basic principle behind atomic absorption spectrometry is that metals in the ground state will absorb radiation from a light beam with the same spectral composition as the light emitted by the element under consideration. Cadmium atoms thus absorb radiation from light emitted by a cadmium lamp. The decrease in intensity as compared to a blank corresponds to the concentration of the metal. There are two main methods for atomization of a sample, the flame method and the furnace method, the latter also being called electrothermal atomic absorption. The flame methods use different mixtures of gases for creating a high-temperature atomization flame, e.g. air-acetylene. Different types of flames may be used for different metals. Flame methods are generally used for liquid samples which can be aspirated directly into the flame. An important modification is the technique developed by Delves (1970) where a small amount of a liquid sample, e.g. blood is put into a microcrucible made of nickel and then inserted in the flame. During the last years, the flameless methods have undergone a rapid development. Most common is the graphite tube furnace. The principle is that after drying in the furnace, the temperature is raised to atomize the sample which then passes through the light path. Several milliliters of solution must be used in the conventional flame method, while only 1-100 μl need be applied to the furnace. The standard solution, preferably in a similar matrix, is treated in the same way. Atomic emission spectrometry has found a number of applications and has been especially useful for the determination of lead, cadmium, and mercury in biological materials and in exposure media as well as for the determination of many essential elements, e.g. copper and zinc. Recently methods have also been developed for nickel and arsenic in different media. Special modifications are required for highly volatile metals such as mercury and arsenic. One limitation is that atomic absorption is not suited for multi-element analysis. Also, some non-specific absorption may occur due to the presence of other atoms and molecules in the flame. Salts such as sodium chloride and phosphates are especially apt to cause interference. This may be avoided by background correction, for which a deuterium lamp is generally used. Many of the modern apparatuses have such a lamp built in. In some cases, this type of background correction does not suffice for all the non-specific absorption. For example, in the direct analysis of cadmium in urine, values obtained will be too high. The next step is then to extract the metal according to similar principles as for colorimetric analysis and to introduce the metal chelate in an organic solvent into the flame or the graphite furnace. The most common chelating agent has been APDC (ammonium pyrolidine dithiocarbamate), which is extracted into MIBK (methyl-iso-butyl-ketone). This can be used for several metals, e.g. cadmium, lead, and nickel.

2.2.1.4 X-ray methods

X-ray methods are relatively new and are not a matter of routine to any large extent. The basic principle is to subject the sample to high intensity X-rays or still better, protons (Johansson, 1971), and to measure the radiation emitted due to electron displacement in the irradiated atoms. The spectral lines of emitted X-rays can be resolved by a crystal. A multi-element analysis is thus possible to perform by this method. A great advantage is that very small amounts can be analyzed; for example, a single hair sample can be subjected to multi-element analysis. More data are needed to compare these methods with the more established procedures before a final verdict can be made.

2.2.2 Neutron activation

Neutron activation analysis is based on the formation of radioactive isotopes when a sample is irradiated by neutrons from a nuclear reactor or from some other neutron source. The element of interest is then identified and quantitated by γ-spectroscopy. Neutron activation analysis has been used mainly for research and has only come into routine work for the determination of total mercury in fish. Even here, its role is on the decline thanks to the development of atomic absorption methods. Neutron activation analysis has been used for multi-element studies, in which cases complicated extraction schemes have often been carried out. It should be noted that lead and tin are not suitable for analysis by that method. Neutron activation is also often used as a reference method for testing the accuracy of other methods since, if properly performed, it has a high specificity and accuracy. Some limitations are that a reactor is needed and that the cost of analysis is high.

2.2.3 Electro-chemical methods

Electro-chemical methods are based on the measurement of changes in electro-chemical potentials, which are due to transfer of electrons from one element to another. These methods use electrodes of different types. For example, in polarography, a dropping mercury electrode is applied. By changing the charge of that electrode, different metals in solution will form amalgams with the mercury at different potentials. In this way, so-called polarographic waves are obtained, from which quantitative estimates can be made of the concentration of different metals. A further development of this method is anodic stripping voltametry which is based upon the reverse process, i.e. metals are first concentrated on the electrode, and then released, whereafter the changes in the potentials are measured. This method has proved suitable for water analysis, but experience is still limited as to its value for biological materials. Its suitability for samples in which it is easy to obtain metals in an ionic form is obvious.

2.2.3 Chromatography

Chromatography is based on differential adsorption of dissolved substances from a suitable solvent (mobile phase) which is passed through a stationary adsorbent (paper, silica gel etc.). Chromatographic methods are of great importance for separating and identifying metal compounds, but for final quantitative analysis only gas chromatography has been routinely used. Chromatographic methods are also used in the pretreatment procedures, e.g. ion exchange has been used in preparation for atomic absorption analysis or for multi-element neutron activation procedures.

In gas liquid chromatography the stationary phase is a column filled with a substance in liquid state that adsorbs organic compounds. The mobile phase is a gas. The sample in an organic solvent is injected into the system and carried by the gas to the column, where components are adsorbed and separated. They will then pass a detector, yielding peak recordings, which enable their quantification. Methylmercury and alkyllead especially have been determined by this method. The determination of alkyllead in gasoline is a relatively simple procedure, since the organic metal compound is already in a solvent, whereas several pretreatment steps must be performed before the methylmercury in fish can be extracted into a solvent.

Very small amounts of alkyllead and alkylmercury can be detected by gas chromatography. It is possible to analyze for other metals by converting them to organic complexes, but this has not yet been done on a routine basis.

2.2.4 Spark source mass spectrometry (SSMS)

The basis for spark source mass spectrometry is the formation of metal ions when metals are subjected to high energy discharges. A beam of metal ions is then directed into a mass spectrometer where they will separate into a magnetic and electrostatic field according to their mass and charge. The mass spectra can be recorded on a photoplate, from which quantitative estimates can be made. All elements from lithium to uranium may be determined. Non-radioactive isotopes can be separated by this method and identified, as has been done in studies on lead metabolism.

Spark source mass spectrometry has been used in several studies on metals in food, blood, organs etc. Its limitations are above all the high cost of the equipment and the high skill required in operating and maintaining it. Like neutron activation analysis, SSMS will surely serve well in the future as a reference and special research method.

2.3 Quality control and inter-laboratory comparisons

The precision of a method can be tested by performing repeated determinations on the same samples, but to ensure accuracy, it is often necessary to go beyond the resources of one's own laboratory and, for example, to use so-called reference samples, among which the most well-known are the NBS (National Bureau of Standards, U.S.A.) standard reference materials. A typical example of an NBS standard for a metal is the bovine liver, which many investigators have used when checking their methods. While the bovine liver is excellent for testing methods for the determination of metals in liver and other tissues with a similar composition, it cannot be used to guarantee accurate results for e.g. blood. Unfortunately, many results have been reported where methods for the analysis of blood have been assessed by using other standards. The only appropriate references for checking the accuracy of a method for determination of metals in blood are blood samples with a known content of the given metal.

Recovery tests are useful when checking for losses etc. in the different steps in an analysis, but should not be used to prove accuracy.

Another way to ensure accuracy for a method is to determine the metal content of a sample with other methods which are based on different principles. Thus determination with neutron activation, and spark source mass spectrometry, should give a good idea of the accuracy of a method using atomic absorption spectrophotometry. When a method has been tested and found to be accurate it should also be subjected to future checks through regular analysis of some standard sample.

The need for accurate analytical methods has stimulated an active interest in collaborative studies, where several laboratories participate. Examples are the studies by Keppler et al. (1970), Lauwerys et al. (1975), and Kjellström et al. (1975). The results of these studies have been disappointing, showing very large differences for different types of samples. Lauwerys et al. (1975) concluded "... several laboratories that took part in this comparison programme have not yet adequately developed the technique required for precisely measuring Pb, Cd and Hg in blood, urine and water." For details on statistical procedures for use in collaborative tests, see Youden (1975) and Steiner (1975).

3. Conclusions

During the last few years, the need for standardizing sampling procedures and for having good programmes for the quality control of analytical results has been recognized. The analytical techniques available today are often extremely sensitive. Before any new method is used in environmental monitoring, it must be checked for accuracy by comparisons with other established methods within and outside one's own laboratory. If the analytical data are not accurate, erroneous conclusions with regard to dose-response may be drawn and these may be highly misleading in the evaluation of health hazards.

REFERENCES

Anand, V.D., White, J.M. and Hipolito, V.N. (1975). Clin. Chem. 21, 595-602.

APHA (1976). "Standard Methods for the Examination of Water and Waste Water." 14th edition, pp 1193. APHA, AWWA and WPCF, American Public Health Association, Washington, D.C.

Azar, A., Snee, R.D. and Habibi, K. (1973). In: "Environmental Health Aspects of Lead." pp 581-594. Published by the Commission of European Communities Directorate General for Dissemination of Knowledge, Center for Information and Documentation, Luxembourg.

CEC-WHO-EPA (1977). "International Workshop on the Use of Biological Specimens for the Assessment of Human Exposure to Environmental Pollutants." To be published.

Coenen, W. (1966). Staub-Reinhalt. Luft 26, 216-221.

Crosby, N.T. (1977). Analyst 102, 225-268.

Delves, H.T. (1970). Analyst 95, 431.

EPA (1972). "Handbook for Analytical Quality Control in Water and Wastewater Laboratories." U.S. Environmental Protection Agency, Technology Transfer by Analytical Quality Control Laboratory, National Environmental Research Center, Cincinnati.

EPA (1976). "Quality Assurance Handbook for Air Pollution Management Systems." U.S. Environmental Protection Agency, Office of Research and Development, Environmetal Monitoring and Support Laboratory, EPA 600/9-76-005, Research Triangle Park.

FAO/WHO (1977). Report of the consultation on the Joint FAO/WHO Food and Animal Feed Contamination Monitoring Programme - Phase II, Geneva.

Friberg, L., Nordberg, G. and Piscator, M. (1977). In: "Toxicology of Metals." Volume II, pp 124-163. Environmental Health Research Series, 600/1-77-022, Environmental Protection Agency, Research Triangle Park.

Goodman, G.T. and Roberts, T.M. (1971). Nature 231, 287-291.

ICRP (1975). Report of the Task Group on Reference Man, ICRP Publication No. 23, Pergamon Press, Oxford.

Johansson, T.B., Akselsson, R. and Johansson, S.A.E. (1971). Nucl. Instrum. Method. 84, 141.

Katz, M. (1969). "Measurement of Air Pollutants." pp 123. World Health Organization, Geneva.

Keppler, J.F., Maxfield, M.E., Moss, V.D., Tietjen, G. and Linch, A.L. (1970). Amer. Ind. Hyg. Assoc. J. 31, 412-429.

Kjellström, T., Tsuchiya, K., Tompkins, E., Takabatake, E., Lind, B. and Linnman, L. (1975). In: "Proceedings of the CEC-EPA-WHO International Symposium on Recent Advances in the Assessment of Health Effects of Environmental Pollution." pp 2197-2213. Published by the Commission of European Communities Directorate General for Dissemination of Knowledge, Center for Information and Documentation, CID, Luxembourg.

La Fleur, P.D., ed. (1976). "Accuracy in Trace Analysis: Sampling, Sample Handling, Analysis." Proceedings of the 7th Materials Research Symposium, Vol. I and II, pp 1304. US. Government Printing Office, Washington, D.C.

Lauwerys,R., Buchet, J.P., Roels, H., Berlin, A. and Smeets, J. (1975). Clin. Chem. 21, 551-557.

Leidel, N.A. and Busch, K.A. (1975). "Statistical Methods for the Determination of Noncompliance with Occupational Health Standards." HEW Publication No. (NIOSH) 75-159. National Institute of Occupational Safety and Health, Cincinnati.

Mahaffey, K.R., Corneliussen, P.E., Jelinek, C.F. and Fiorine, J.A. (1975). Environ. Health Perspect. 12, 63-69.

Maienthal, E.J. and Becker, O.A. (1976). Interface 5, 49-61.

Mertz, W. (1975). Clin. Chem. 21, 468-475.

NIOSH (1975). "Manual of Analytical Methods." U.S. Department of Health, Education and Welfare, HEW Publication No. (NIOSH) 75-1211, Cincinnati.

Pecsok, R.L., Shields, L.D., Cairns, T. and McWilliam, I.G. (1976). "Modern Methods of Chemical Analysis." 2nd edition, pp 573. John Wiley and Sons, New York.

Piscator, M., Kjellström, T. and Lind, B. (1976). Lancet 2, 587.

Rühling, Å. and Tyler, G. (1973). Water Air Soil Pollut. 2, 445-455.

SCOPE (1975). "Environmental Pollutants - Selected Analytical Methods." pp 277. Butterworths, London.

Schrauzer, G.N., White, O.H. and Schneider, C.J. (1977). Bioinorg. Chem. 7, 35-56.

Sherwood, J. (1971). Amer. Hyg. Assoc. J. 32, 810-816.

Steiner, E.H. (1975). In: "Statistical Manual of the Association of Official Analytical Chemists." pp 65-88. Washington, D.C.

Tipton, I.H., Cook, M.J., Steiner, R., Boyes, C.A., Perry, H.M. and Schroeder, H.A. (1963). Health Phys. 9, 89-102.

Ulfvarson, U. (1977). Scand. J. Work Environ. Health 3, 109-115.

WHO (1977). "Air Monitoring Programme Design for Urban and Industrial Areas." pp 46. WHO Offset Publication No. 38, World Health Organization, Geneva.

Winefordner, J.D., ed. (1976). "Trace Analysis - Spectroscopic Methods for Elements." Chemical Analysis Series, Vol. 46, John Wiley and Sons, New York.

Youden, W.J. (1975). In: "Statistical Manual of the Association of Official Analytical Chemists." pp 1-63. Washington, D.C.

Zoeteman, B.C.J. and Brinkmann, F.J.J. (1976). In: "Hardness of Drinking Water and Public Health." pp 173-202. Pergamon Press, Oxford.

SOURCES, TRANSPORT, AND TRANSFORMATION OF METALS IN THE ENVIRONMENT

Karin Beijer and Arne Jernelöv

1. Sources

Some of the most important natural sources of metals in the atmosphere are the release from surface waters, soils and vegetation, volcanic activity and forest fires. Metals in the surface waters originate to a large extent from bedrock, surface run-off and atmospheric deposition. The major anthropogenic sources for metals in the environment are the combustion of fossil fuels, mining and smelting operations, processing and manufacturing industries, farming and forestry (fertilizers and pesticides; increased soil erosion) and waste disposal (dumping, release of domestic sewage, scrap metal handling etc).

The world production of metals is shown in Table 1. The data on the volume and increase/decrease of the production of a metal may give an indication of the extent of its possible mobilization in the environment and thereby the pollution problem involved. Table 2 lists the uses of some metals. A very large part is used in the production of alloys and in plating. The chemical industry utilizes an increasing amount of metals as catalysts. In the production of plastics considerable amounts are used e.g. as heat stabilizers. Large quantities of metals also figure in pigments, biocides and lubricants and in the ceramic and electrical industries. Fertilizers and pesticides often contain metals.

Table 3 lists the estimated total emission of metals from oil combustion during 1971 in Sweden. Combustion of fossil fuels and production of cement give rise to a mobilization of certain elements, notably mercury and boron, to the oceans via the atmosphere in amounts comparable to those moving to the oceans via rivers in the major sedimentary cycle (Goldberg, 1976). For some metals, viz. arsenic, lead, selenium and zinc, the contribution from cement production seems to be greater than that from the burning of fossil fuels. The increase in European coal production to parallel the increase of heavy metals in the sediments is found to start at the time of the Industrial Revolution.

2. Transport

The amount of material moved about the earth's surface due to the activities of man has been estimated to be about one tenth of that involved in the major weathering cycle (Goldberg, 1972). The major transport routes for metals in the environment are summarized in Figure 1.

2.1 Atmospheric transport

Metals emitted into the atmosphere from the various sources will be carried greater or shorter distances with the winds depending on their state - gaseous, vapor or particulate - before they are deposited on land or on the surface of the oceans through dry fallout or washout with precipitations. In the case of particulates, particle size is the most decisive factor. The distance a metal will be carried is also dependent on meteorological conditions such as winds and precipitation, topography and vegetation. Investigations indicate that most of the metals associated with particles are deposited within 10 km from the point of emission.
The gaseous phase is of course of importance for the aerial transport of mercury but also for that of arsenic, cadmium, lead, antimony, selenium and zinc (Goldberg, 1974; Lövblad and Grennfelt, 1977). An enrichment on the finer particles has been found for the latter elements. Approximately half of the amount of the lead emitted with automobile exhausts is deposited within 100 m, and a third, which is emitted in the form of very fine particles, is transported over long distances (Folkesson, 1976). Mercury behaves differently from other elements due to its very high vapor pressure. It has been shown that the final deposition of about two thirds of emitted mercury takes place within 200-2,000 km. It has also

been shown that airborne mercury strongly interacts with its surroundings. On the average, less than 20% is finally deposited while the rest is re-emitted to the atmosphere (Högström and Enger, 1977; Svedung, 1977).

2.2 Aquatic transport

The greater part of the metal load emitted into the environment is transported by water (lead is an exception). Most of it will eventually reach the lakes and the coastal zones of the oceans via river transport. In a lake the portion of the total metal load carried on particles of different types will settle in areas with active sedimentation and thus be deposited in the sediments from which the metals may be re-released due to microbial activity and changes in various physical and chemical parameters, including pH and redox potential. Very little will ever travel beyond the coastal waters of the oceans and the metal levels decrease with an increasing distance from a river mouth. Two mechanisms caused by increasing salinity and microbial activity may account for this: 1) Salting out of the large molecular weight organic fraction - e.g. humic acids of fresh water - and flocculation of inorganic matter, resulting in increased particle size, will remove absorbed and incorporated metals to the sediment (Matson, 1968). 2) A mobilization from carrier particles by chlorine ions and chelating substances resulting in an increasing availability to the biota. Aquatic organisms will concentrate metals from the ambient water to levels far exceeding the metal levels in the water and thus to a large extent retain them within the biologically active coastal waters.

The mechanisms of the transport of metals have been studied in rivers and estuaries (e.g. Kharkar et al., 1968; Cranstone and Buckley, 1972, Skei et al., 1973; Gibbs, 1973; Landner et al., 1977; Duinker and Nolting, 1977). These studies have clearly shown that a considerable portion of the metals in the water is associated with suspended particles. The extent of this association varies greatly with the metals, the properties of the particles, and the type of water.

2.3 Biological transport

The transport of metals via living organisms also plays a role in the total transport. In Figure 3 the flow of methylmercury and some factors influencing it in a simplified limnic food-chain are outlined. The figure is included to illustrate the complexity of an ecological system even when simplified to this extent. This type of biological transport mostly does not represent any major relocation of the metal in space. Naturally migrating organisms transport their content of metals, but the proportion of the total metal content in an ecosystem that is removed or introduced this way is usually small. For an understanding of the biological response, however, the processes are essential.

One type of situation where "biological transport" does influence metal transport in a quantitatively important way is in plankton in coastal areas. High standing crops of phyto- and zooplankton may absorb a large part of the metals brought to the coastal zone via rivers. Included in fecal pellets or in dead organisms, the metals may then settle and become incorporated in sediments instead of being transported further out into the oceans.

3. Chemical form and transformation

For an understanding of the effects of metal pollution it is important to gain a thorough knowledge of the transformation undergone by the metals from one chemical species to another.

3.1 Physical - chemical transformation

Metals in the aquatic environment may exist in the following forms: 1) soluble: a) free hydrated ions; b) complex and chelate ions with inorganic ligands including OH^-, CO_3^{2-}, Cl^- or organic ligands including amines, proteins, humic and fulvic acids; c) organic molecules. 2) particulate: a) colloidal complexes or aggregates of e.g. hydrated oxides; b) adsorbed onto different types of particles; c) precipitated, e.g. as metal coatings on particles; d) incorporated in organic particles, plankton etc.; e) held in the structural lattice in crystalline detrital mineral particles. The speciation is controlled by environmental variables such as pH; redox potential - dissolved oxygen, ionic strength - salinity, alkalinity and hardness, the presence of organic and particulate matter, and biological activity, in addition to the intrinsic properties of the metals.

The transformation between the different chemical form is induced by changes in these variables. For example, the lowering of pH (as will for instance occur when a river with a neutral pH enters an acidified lake), will cause a release of metals from complexes and particulate matter; a mobilization of metals bound in sulfides in anoxic sediments will occur upon the introduction of oxygen; metals associated with hydrated iron oxide will be released under reducing conditions.

3.2 Redox transformations

In living organisms enzyme systems have evolved that are capable of changing the oxidation states of chemical compounds. In an environment rich in microbial activity each oxidation state might be made available for metabolic interconversions. Woolfolk and Whiteley (1962) gave several references to work dealing with microbial reductions up to 1962. A lot of research has been done in this field since. Only a few examples will be mentioned here. At least fifteen strains of bacteria have been found to oxidize arsenite to arsenate in cattle dip solutions. Specific enzymatic activity (arsenite dehydrogenase) was found in purified preparations (Turner and Legge, 1954). The reduction of arsenate to arsenite occurs in cultures of the yeast *Pichia quillermondi* (Bautista and Alexander, 1972), *Chlorella* (Blasco et al., 1972) and marine bacteria (Johnson, 1972), while the oxidation of arsenite to arsenate is brought about by *Pseudomonas sp.* (Turner, 1949).

A plasmid[x)] - specified mercury reducing system will reduce both silver (I) and gold (III) to their elemental state. The reducing system is induced only by mercury, not by gold or silver, however (Summers and Sugarman, 1974).

The oxidation of molecular hydrogen by extracts of Micrococcus lacticytilus at the expense of certain chemical species of arsenic, bismuth, selenium, tellurium, lead, thallium, vanadium, manganese, iron, copper, molybdenum, tungsten, osmium, ruthenium, gold, silver and uranium as well as molecular oxygen has been reported (Woolfolk and Whiteley, 1962). Theobacillus ferro oxidans is able to oxidize ferrous ion to ferric at pH 2 in mixed sulfide ores (Tuovinen and Kelly, 1974). A culture of Schizosaccaromyces, supplied with tellurite ions, will carry out a reduction which brings the metal to its elementary stage (Smith, 1974).

3.3 Formation and degradation of organic compounds

Many metals and metalloids are released into the environment in relatively non-toxic forms. They may subsequently acquire an enhanced toxicity as organometals through environmental interactions involving both biological and non-biological processes. Such pathways may potentially combine, serving then as a means for metal transport, particularly across sediment-water-organism interfaces.

3.3.1 Methylations of metals and metalloids

That arsenic, selenium and tellurium can undergo biological methylation has long been recognized. A comprehensive review of the knowledge concerning biological methylation up to 1945 was made by Challenger (1945). Inorganic compounds of of arsenic and selenium were shown to be methylated within Scropulariopsis brevicaulis and tellurium compounds were

---

x) Plasmids - small ancillary circular DNA molecules carrying extra chromosomal genes often specifying bacterial resistance to toxic substances.

predicted to follow the same metabolic pathway in the organisms. Some Penicillum strains were also shown to methylate selenite and tellurite ions when these ions were present together in bread cultures. At the time, there was no evidence of bacterial methylation.

Very little new information on biological methylation of metal compounds was generated until the mid-sixties. Westöö at that time demonstrated (1966) that mercury in fish is predominantly present in the form of methylmercury. It was soon shown that some microorganisms, unidentified, in natural organic lake sediment could methylate mercury (Jensen and Jernelöv, 1967). The net result of the process was mono- or dimethylmercury and the rate of biological methylation of mercury was found to be well correlated with general microbiological activity in the sediment (Jensen and Jernelöv, 1969).

Methylvitamin B_{12} has been shown to be capable of transferring methyl groups to mercury (II), thallium (III), platinum (II) and gold (I) in vitro (Agnes et al., 1971a, 1971b). Two different pathways seem to be involved. The one, which mercury (II) and thallium (III) follow, is apparently an acid-base reaction. The other, which platinum and gold follow, is probably an oxidation-reduction "redox switch" reaction, since it requires both platinum (II), and platinum (IV) or both gold (I) and gold (III). Cadmium (II), lead (II) and indium (III) were found to be ineffective. In each case the first step of the reaction is the production of the methylated metal. In the second step the methyl group can readily be transferred further to a good nucleophile. All methylated metal compounds except methylmercury readily decompose to metal ions and methyl groups.

Methylcobalamin has been implicated in the methylation of mercury, lead, tin, pallacium, gold, platinum and thallium as well as arsenic, selenium, tellurium and sulfur (Wood et al., 1977). According to Wood and his co-workers, two general mechanisms exist for the methylation by methylcobalamin: 1) reactions in which the metal or metalloid acts as an electrophile; 2) reactions in which the metal or metalloid acts to abstract a methyl radical. Type 1 reactions involve a transfer of CH_3^- to the more oxidized state of the element, and type 2 a transfer of $CH_3\cdot$ to the reduced member of a redox couple. It appears that those elements which react according to the type 1 mechanism (Tl, Pd, Hg) have redox potentials greater than + 0.8 volts, and that the more oxidized member of the redox couple, Tl (III), Pd (II) and Hg (II), is the species which is methylated by methyl-B_{12}.

Those elements which are known to react via the type 2 mechanism, e.g. Cr (II), are found at the reducing end of the redox potential scale. Elements of intermediate redox potential react via the "redox switch" mechanism described by Agnes et al. (1971a, 1971b).

3.3.1.1 Methylation of mercury

The biological cycle of mercury is shown in Figure 3. That microbial activity is a prerequisite for the synthesis of methylmercury under natural conditions, unless other methylated metal compounds are present, has been shown in several experiments. Bacteria isolated from mucous material on the surface of fish have been shown to be able to methylate mercury (Jernelöv, 1968). Extracellular methylmercury formation has been demonstrated for several bacteria (Kitamura et al., 1969; Yamada and Tonomura, 1972; Vonk and Sijpersteijn, 1973) and intracellular methylmercury formation has been demonstrated for several fungi (Landner, 1971; Vonk and Sijpersteijn, 1973). Methylation *in vivo* was studied in aerobic cultures of *Neurospora crassa*. Mercury-tolerant mutants brought about methylation very effectively when an excess of cysteine or homocysteine was present in the substrate. It has been suggested that the biomethylation might be an "incorrect" synthesis of methionine - normally formed through methylation of homocysteine (Landner, 1971).

The potential for microbial methylation of mercury by fungi and bacteria has been shown to exist under aerobic as well as anaerobic conditions. The ecological significance of these findings, especially of the anaerobic methylation, is difficult to evaluate, however. For instance, methylcobalamin is known to be unstable in a natural environment. The transmethylating activity of methylcobalamin in vitro was found to be inhibited by cellular proteins and thiol groups (Bertilsson and Neujahr, 1971). Since mercury is hardly present in nature under anaerobic conditions without the simultaneous presence of hydrogen sulfide, mercuric sulfide is likely to be formed. Under these conditions mercury will be effectively prevented from being methylated.

No formation of methylmercury was found in anaerobic mud in some experiments by Rissanen (1974), probably due to the existence of mercuric sulfide. It is true that the sulfide will be oxidized to sulfate, if aerobic conditions should be re-established, but this oxidation is probably slow. The methylation rate was found to be 100-1000 times slower in aerobic sediments with mercuric sulfide as the mercury donor as compared with mercuric chloride (Fagerström and Jernelöv, 1971). In fish allowed to accumulate mercury from sediments, mercuric sulfide was very slowly mobilized as compared to mercuric chloride (Gillespie, 1972).

The existence of a demethylating capacity, which was first described in 1969 (Furukawa et al., 1969; Kitamura et al., 1969), is a complicating factor when attempts are made to transfer laboratory results to the ecosystem level. Most experiments on rates of methylation have been performed in such a way as not to allow for a distinction between the two competing processes, methylation and demethylation. Accordingly, it is possible that most of the results that have been interpreted as measures of gross methylation rates have in fact been net methylation rates. The kinetics of the response of such a competitive system in relation to external stimuli, such as temperature, is of course more complicated, rendering the interpretation of data more uncertain than if methylation alone is presupposed to occur.

Bisogni and Lawrence (1973) studied the net methylation with regard to the effects of redox potential, inorganic mercury concentration, temperature, microbial activity and sulfide concentration. A proposed kinetic model showed: 1) Monomethylmercury is the predominant product of methylation of mercury (near neutral pH); 2) The rate of methylation is higher in aerobic than in anaerobic systems for a given inorganic mercury concentration and microbial growth rate; 3) Higher microbial growth produces higher methylation rates under aerobic as well as anaerobic conditions; 4) Methylation rates can be reduced by the addition of sulfide to some anaerobic systems; 5) Temperature affects methylation rates in accordance with its effects on the metabolic rate of the methylating organism.

3.3.1.2 Methylation of other metals and metalloids

The methylation of the three metalloids has been proposed to follow the same metabolic pathways (Challenger, 1945; McBride and Wolfe, 1971; Wood, 1974).

Many investigators have reported the microbial formation of methylated species of arsenic, selenium and tellurium. The production of dimethylselenide from inorganic selenium compounds by several types of fungi has been shown (Challenger, 1945; Fleming and Alexander, 1972; Burlees and Flemming, 1974). Anionic forms of selenium and tellurium are subject to microbial alkylation, the products being dimethylselenide and dimethyltelluride (Burlees and Flemming, 1974).

A coryneform bacterium has been reported to convert inorganic selenium to Me_2Se_2 (Francis et al., 1974). Three bacterial isolates from lake sediment were able to convert sodium selenite to Me_2Se, Me_2Se_2 and an unknown volatile selenium compound during their growth (Chau et al., 1976). Some fungi isolated from industrial and agricultural arsenic-containing sludge, e.g. Candida humicola, are capable of converting salts of arsenic, arsenous monomethylarsenic or dimethylarsonic acids to trimethylarsine (Cox and Alexander, 1973a, 1973b).

Selenium and tellurium as well as arsenic are readily methylated by the methanogenic bacteria Methanobacterium, strain M.O.H., under anaerobic conditions. The inorganic arsenic forms were reduced and gradually methylated to dimethylarsine. Dimethylarsinic acid and methylarsonic acid were intermediates (McBride and Wolfe, 1971). Fresh water, seawater, eggshells and rock samples were analyzed for arsenic(V), arsenic(III), methylarsonic acid and dimethylarsonic acid. The methylated compounds were found in most biological samples but not in limestone (Braman and Foreback, 1973).

Evidence has been put forth that microorganisms in lake sediments can transform certain inorganic and organic lead compounds into the volatile tetramethyllead (Me_4Pb). Me_3Pb^+ was converted to Me_4Pb by bacterial isolates from Lake Ontario. Species of Pseudomonas, Alcaligeneus, Actinetobacter, Flavobacterium and Aeromonas could transform Me_3PbOAc into Me_4Pb. None of the isolates could produce Ne_4Pb from inorganic lead (Wong et al., 1975). Tin has also been demonstrated to undergo biological methylation (Huey et al., 1974).

3.3.1.3 Photoalkylation

In the presence of acetate ion or acetic acid in aqueous solutions, mercury(II) may be photoalkylated if exposed to sunlight (Agaki and Takabatake, 1973). Photolysis of mercury chloride in the presence of sodium acetate under normal laboratory fluorescent lighting has been found to produce methylmercury ion, dimethylmercury and metallic mercury (Jewett et al., 1975). A methyl-chromium bond has been found to be formed in the long-lived methylpentaaquochromium(III) ion during the photolysis of tertiary-butoxy radicals and chromium(II) in aqueous solution (Ardon et al., 1971).

3.3.1.4 Transalkylation

Abiotic transalkylation between several metals in aqueous solution has been demonstrated (Jewett and Brinkman, 1974) and is shown in Table 4. Example of reactions:

$$(CH)_3Sn^+ + Hg^{2+} \rightarrow (CH_3)_2Sn^{2+} + CH_3Hg^+$$

$$(CH_3)_3Sn^+ + PtCl_4^{2-} \rightarrow \{CH_3PtCl_3^-\} \rightarrow C_2H_6 + Pt^o + (CH_3)_2Sn^{2+}$$

Trimethyltin and trimethyllead seem to display the same transmethylation chemistry. Trimethyllead is a far more rapid methylator of mercury(II), though, being comparable to methylcobalamin in its ability to transfer CH_3 to mercury. The methylation of the noble metals palladium(II), gold(III) and platinum(II) proceeds readily but the reaction pathway is quite different. Methylmercury can also act as an effective methyl donor for the noble metals although the reaction rate is considerably slower.

A species of Pseudomonas which produces a volatile methylated tin species from tin(IV) has been isolated from the Chesapeake Bay (Huey et al., 1974). The same organism is also quite active in forming elemental mercury from phenylmercuric acetate and mercuric ion. But as was shown, in the presence of both mercury(II) and tin(IV), methylmercury is formed by this organism through a mixed biotic and abiotic process. An abiotic transalkylation process between alkyllead compounds and inorganic mercury has been demonstrated in the St. Clair River and in the laboratory, in sterilized sediments (Jernelöv et al., 1972).

In experiments investigating methylation of selenium by sewage fungi some Penicillum strains were isolated from the sewage. When tellurium compounds were added ($TeCl_4$, H_2TeO_2 and H_6TeO_6), they were found to be methylated as effectively as selenium compounds, but only in the presence of selenium. The yield of dimethyltelluride was proportional to the input of inorganic selenium and methylated tellurium was found until the input ratio of selenium and tellurium was about 10:1. This indicates a possible transalkylation, and not a direct biological alkylation, of tellurium (Fleming and Alexander, 1972).

3.3.2 Demethylation of mercury

The biological stability of organometallic compounds varies widely. Some of them decompose rapidly through non-enzymatic processes or through the action of non-specific enzymes. Others are highly persistent and are degraded only through specific biochemical processes. Short-chain alkylmercury compounds belong to the latter group.

Decomposition of organomercurials by bacteria, resulting in the formation of elemental mercury, has been reported by several investigators. This process was first observed in Japan when bacteria isolated from soil heavily contaminated with organomercurials were found to be capable of converting phenylmercury and methylmercury into metallic mercury and benzene and methane respectively (Tonomura et al., 1968). A large number of bacterial strains, and also yeasts, resistant to and capable of hydrolyzing and reducing mercury compounds have been found since, some of which have been investigated more thoroughly (e.g. Spangler et al., 1973; Brunker and Bott, 1974; Billen et al., 1974; Blair et al., 1974; Colwell and Nelson, 1975).

Two distinct groups of organisms have been observed: those resistant to organomercurials as well as mercury(II), and those resistant to mercuric ion only. The cleavage of the mercury-carbon bond in organic mercury compounds and the reduction of mercury(II) have invariably been found to be catalyzed by enzyme systems - either inducible or constitutive - one or more to catalyze the cleavage of the mercury-carbon bond of different organic compounds and one to catalyze the reduction of the mercuric ion formed in the first reaction or encountered in the environment. In some cases the work on purifying and characterizing the different enzymes has progressed very far (Furukawa and Tonomura, 1972; Izaki et al., 1974; Tezuka and Tonomura, 1976; Schottel and Silver, 1976). In one case, two different hydrolases with different physical properties have been isolated from bacteria. The differences in their enzymatic properties are not yet fully understood, however (Tezuka and Tonomura, 1976). In another case at least two hydrolases have been found and purified in a mutant of Escherichia coli, one active against methylmercury, the other against phenylmercury (Schottel and Silver, 1976). When the genetic background for the resistance has been worked out, it has been found to be associated with plasmids (Summers and Lewis, 1973; Schottel et al., 1974; Silver et al., 1975).

3.3.3 Combined effects of alkylation and dealkylation

It has been suggested that an equilibrium may be reached between the production (addition) of methylmercury and its degradation in mercury polluted environments. Methylmercury degrading activity by bacteria in sediments from the Baltic Sea has been demonstrated, as later described by Jernelöv et al. (1975). In the light of these findings an earlier study on methylation of mercury in the St. Clair system on the Canada/USA border was re-evaluated. The results were demonstrated to support the idea of an equilibrium between chemically alkylated (from methyl- and ethyllead) mercury and biological degradation (Jernelöv et al., 1972).

The hypothesis can be formulated that in a sediment, under a constant flow of methylmercury into and/or out of the system, biological formation and degradation of methylmercury will result in an equilibrium, with a constant level of methylmercury in the sediment. This is a result of the fact that the formation is concentration-dependent with regard to inorganic mercury and the degradation is concentration-dependent with regard to methylmercury. If input or output of methylmercury is varying, a disrupted pattern will result, where the methylmercury level in the sediment tends to return to the equilibrium as soon as the disturbance has come to an end. The curve in Figure 4a illustrates such a situation. It is obvious that as long as no methylmercury is added, the importance of the demethylating activity will depend upon the relation between methylating activity and the transport of methylmercury out of the sediment - i.e. whether or not this relation is such that the equilibrium is approached.

Transport of methylmercury out of the system can be so great as to prevent methylmercury from building up to concentrations where demethylation becomes important. Such a situation is depicted in Figure 4b. Here, the difference between gross and net methylation rates is of little relevance.

The transport of methylmercury out of the system may be low, whereupon the demethylating activity assumes importance for the regulation of the methylmercury concentration in the sediment (see Figure 4c). In this case the difference between gross and net methylation rates is large.

In short, the concentration of methylmercury in the sediment is low when the amount transported out of it is large and vice versa. Thus, in this example, a negative correlation exists between methylmercury concentration in the system, in this case, sediments, and the amount of methylated mercury released from the system through biological demethylation.

REFERENCES

Agaki, H. and Takabatake, E. (1973). Chemosphere 1, 131-133.

Agnes, G., Bendle, S., Hill, H.A.O., Williams, F.R. and Williams, R.J.P. (1971a). Chem. Commun. pp 850-851.

Agnes, G., Hill, H.A.O., Pratt, J.M., Ridsdale, S.C.R., Kennedy, F.S. and Williams, R.J.P. (1971b). Biochem. Biophys. Acta 252, 207.

Andersson, G. and Grennfelt, P. (1973). "Determination of heavy metals in fuel oil and an estimation of emission of heavy metals from oil combustion." Swedish Water and Air Pollution Research Institute, Stockholm (In Swedish, abstract in English).

Ardon, M., Woolmington, K. and Pernick, A. (1971). Inorg. Chem. 10, 2812.

Bautista, E.M. and Alexander, M. (1972). Soil Sci. Soc. Amer. Proc. 36, 918-920.

Bertilsson, L. and Neujahr, H.Y. (1971). Biochemistry 10, 2805-2808.

Billen, G., Joiris, C. and Wollast, R. (1974). Water Res. 8, 219-225.

Bisogni, J.J. and Lawrence, A.W. (1973). Cornell Univ. Tech. Rep. No. 63. Ithaca, N.Y.

Blair, W., Iverson, W.P. and Brinkman, F.E. (1974). Chemosphere 3, 167-174.

Blasco, F., Robert, J.J. and Gaudin, C. (1972). C.R. Acad. Sci. Ser. D. 275, pp 1223-1226.

Braman, R.S. and Foreback, C.C. (1973). Science 182, 1247-1249.

Brunker, R.L. and Bott, T.L. (1974). Appl. Microbiol. 27, 870-873.

Bryan, G.W. (1977). In: "Marine Pollution." (R. Johnston, ed) pp 185-302. Academic Press, London.

Burlees, L. and Flemming, R.W. (1974). Bull. Environ. Contam. Toxicol. 12, 308.

Challenger, F. (1945). Chem. Rev. 36, 315-364.

Chau, Y.K., Wong, P.T.S., Silverberg, B.A., Luxon, P.L. and Bengert, G.A. (1976). Paper presented at "Microbial Transformation of Heavy Metals Workshop." May, 1976. National Bureau of Standards, Washington, D.C.

Colwell, R.R. and Nelson, J.O. (1975). Microbiol. Ecol. 2, 191-218.

Cox, D.P. and Alexander, M. (1973a). Bull. Environ. Contam. Toxicol. 9, 84-88.

Cox, D.P. and Alexander, M. (1973b). Appl. Microbiol. 25, 408-413.

Cranstone, R.E. and Buckley, D.E. (1972). Environ. Sci. Technol. 6, 274-278.

Duinker, J.C. and Nolting, R.F. (1977). Mar. Pollut. Bull. 8, 65-71.

Fagerström, T. and Jernelöv, A. (1971). Water Res. 5, 121-122.

Fagerström, T. and Jernelöv, A. (1972). Water Res. 6, 1193-1202.

Fleming, J.F. and Alexander, L.T. (1972). Appl. Microbiol. 24, 424-429.

Folkeson, L. (1976). "Lead, Especially Exhaust Lead, in the Terrestrial Environment: Accumulation and Ecological Effects, a Literature Survey." 103 pp. Report No. 794, National Swedish Environmental Protection Board, Solna (In Swedish with English summary).

Francis, A.J., Duxburg, J.M. and Alexander, M. (1974). Appl. Microbiol. 28, 248.

Furukawa, K. and Tonomura, K. (1972). Agric. Biol. Chem. 36, 217-226.

Furukawa, K., Suzuki, T. and Tonomura, K. (1969). Agric. Biol. Chem. 33, 128-130.

Gibbs, R.J. (1973). Science 180, 6-7.

Gillespie, D.C. (1972). J. Fish Res. Board Can. 29, 1035-1041.

Goldberg, E.D. (1972). "The Changing Chemistry of the Oceans." pp 267-288. Wiley-Interscience, New York.

Goldberg, E.D. (chairman). (1974). In: "Ecological Toxicology Research." (A.D. McIntyre and C.F. Mills, eds) pp 233-285. Plenum Press, New York.

Goldberg, E.D. (1976). "Health of the Oceans." UNESCO Press.

Huey, C., Brinkman, F.E., Grim, S. and Iverson, W.P. (1974). In: "Proceedings of the International Conference on Transport of Persistant Chemicals in Aquatic Ecosystems." pp II:73-78, May 1-3, 1974. Ottawa.

Högström, U. and Enger, L. (1977). Unpublished data.

Izaki, K., Tashiro, Y. and Funaba, T. (1974). J. Biochem. Tokyo 75, 591-599.

Jensen, S. and Jernelöv, A. (1967). Nord. Forsk. Bioci. Inf. 10, 4 (In Swedish).

Jensen, S. and Jernelöv, A. (1969). Nature 223, 753-754.

Jernelöv, A. (1968). Vatten 24, 360 (In Swedish).

Jernelöv, A., Landner, L. and Larsson, T. (1975). J. Water Pollut. Control Fed. 47, 810-822.

Jernelöv, A., Lann, H., Wennergren, G., Fagerström, T., Åsell, B. and Andersson, R. (1972). Analysis of methylmercury concentrations in sediment from the St. Clair River system. Swedish Water and Air Pollution Research Institute, Stockholm (In Swedish with abstract in English). Unpublished report.

Jewett, K.L. and Brinkman, F.E. (1974). Preprints of papers. Div. Environ. Chem., Amer. Chem. Soc. 14, 218-225.

Jewett, K.L., Brinkman, F.E. and Bellama, J.H. (1975). In: "Symposium on Marine Chemistry in the Coastal Environment." (T. Church, ed) No. 21. American Chemical Society, Washington, D.C.

Johnson, D.L. (1972). Nature 240, 44-45.

Khakar, D.P., Turekian, K.K. and Bertine, K.K. (1968). Geochim. Cosmochim. Acta 32, 285-298.

Kitamura, S., Sumino, K. and Taina, M. (1969). Jap. J. Hyg. 24, 76-77.

Landner, L. (1971). Nature 230, 452-454.

Landner, L., Grahn, O. and Sjöö, L. (1977). Metal transport in three Swedish rivers. Swedish Water and Air Pollution Research Institute, Stockholm (To be published).

Lövblad, G. and Grennfelt, P. (1977). Heavy metals and other trace elements in coal and their atmospheric emission from the combustion of coal - a literature survey. Swedish Water and Air Pollution Research Institute, Stockholm (In Swedish).

Matson, W.R. (1968). "Trace Metals, Equilibrium and Kinetics of Trace Metal Complexes in Natural Media." Diss., Massachusetts Institute of Technology, Cambridge.

McBride, B.C. and Wolfe, R.S. (1971). Biochemistry 10, 4312-4319.

"Minerals Yearbook." (1971). U.S. Bureau of Mines, Washington, D.C.

Rissanen, K. (1974). Personal communication.

Schottel, J., Mandal, A., Toth, K., Clark, D. and Silver, S. (1974). In: "Proceedings of the International Conference on Transport of Persistant Chemicals in Aquatic Ecosystems." pp II:65-71, May 1-3, 1974, Ottawa.

Schottel, J. and Silver, S. (1976). Personal communication.

Silver, S., Schottel, J. and Weiss, A. (1975). In: Proceedings of the 3rd International Biodegradation Symposium." (J.M. Scharpley, ed) Aug. 1975. Kingston, R.I.

Skei, J.M., Price, N.B. and Calvert, S.E. (1973). Ambio 2, 122-124.

Smith, D.G. (1974). J. Gen. Microbiol. 83, 389-392.

Spangler, W.J., Spiragelli, J.L., Rose, J.M., Flippin, R.R. and Miller, H.H. (1973). Appl. Microbiol. 25, 488-493.

Summers, A.O. and Lewis, E. (1973). J. Bacteriol. 113, 1070-1072.

Summers, A.O. and Sugarman, L.I. (1974). J. Bacteriol. 119, 242-249.

Svedung, I. (1977). Unpublished data.

Tezuka, T. and Tonomura, K. (1976). J. Biochem. 80, 79-87.

Tonomura, K., Nakagami, T., Futai, F., Maeda, K. and Yamada, M. (1968). Nature 217, 644-646.

Tuovinen, O.H. and Kelly, D.P. (1974). Int. Metall. Rev. 19, 21.

Turner, A.W. (1949). Nature 164, 76-77.

Turner, A.W. and Legge, J.W. (1954). Aust. J. Biol. Sci. 7, 452.

Vonk, J.W. and Kaars Sijpersteijn, A. (1973). Antonie van Leeuwenhoek 39, 505-513.

Westöö, G. (1966). Acta Chem. Scand. 20, 2131-2137.

Wong, P.T.S., Chau, Y.K. and Luxon, P.L. (1975). Nature 253, 263-264.

Wood, J.M. (1974). Science 183, 1049-1054.

Wood, J.M., Kennedy, S. and Rosen, G.G. (1968). Nature 220, 173-174.

Wood, J.M., Ridley, W.P., Dizikes, L.J., Cheh, A.M. and Frick, T.D. (1977). Paper presented at the International Symposium on "Factors Influencing Susceptibility to Metal Toxicity." Scientific Committee on the Toxicology of Metals, Stockholm, July 17-22, 1977.

Woolfolk, C.A. and Whiteley, H.R. (1962). J. Bacteriol. 84, 647-658.

Yamada, M. and Tonomura, K. (1972). J. Ferment. Technol. 50, 159-166.

Table 1. World production (metric tons) of metals (From Minerals Yearbook, 1971).

Metal	1965 Production	1969 Production	Increase	Comment
Ag	8 000	9 000	12.1 %	
Al	6 300 000	9 000 000	44.2 %	Al
As	59 000	52 000	-11.4 %	Irregular production
Au	1 400	1 400	0 %	Irregular production
B		700 000		Estimated as B_2O_3
Ba	3 500 000	3 800 000	7.3 %	$BaSO_4$
Be	5 600	7 200	29.5 %	
Bi	3 000	3 800	29.7 %	
Ca		560 000		Per annum USA
Cd	12 000	17 000	42.9 %	
Ce	7 200	11 700	62.7 %	Monazite, $CePO_4$ incl. lanthanides
Co	18 000	20 000	9.8 %	
Cr	4 800 000	5 100 000	6.2 %	Chromite
Cs		242		Import USA
Cu	5 000 000	6 000 000	19.3 %	Ore
	5 500 000	6 600 000	19.3 %	Metal production
Dy				
Er				
Eu				
Fe	620 000 000	720 000 000	16.4 %	Fe in prod. ore
Ga		1.21		Import USA
Gd				
Ge		3.56		Import USA
Hf		0.2		Import USA
Hg	9 200	9 900	6.6 %	
Ho				
In		8.81		Import USA
Ir				See platinum group
K	14 000 000	17 000 000	21.2 %	
La				
Li	8 600			Argentina, Australia, Brazil and South Africa only

Table 1. (Cont'd).

Metal	1965 Production	1969 Production	Increase	Comment
Lu				
Mg	160 000	200 000	24.1 %	
Mn	17 000 000 (1967)	18 000 000	5.9 %	
Mo	45 000	65 000	44.9 %	USSR, Eastern Europe, China excluded
Na	120 000 000	130 000 000	12.6 %	As NaCl
Nb				See Ta
Nd				
Ni	430 000	480 000	13.2 %	
Os				
Pb	2 700 000	3 200 000	19.0 %	Ore
	2 600 000	3 300 000	26.0 %	Metal production
Pd	92	105	13.8 %	Platinum group
Pr				
Pt		6.95		
Rb				
Re		9.1		Consumption per year est. 1972 USA
Rh				
Ru				See Platinum group
Sb	63 000	65 000	3.8 %	Irregular increase
Sc				
Se	820	1 250	53.0 %	
Sm				
Sn	200 000	230 000	11.3 %	Ore
	200 000	230 000	14.0 %	Metal production
Sr	10 000	27 000	170.0 %	As $SrSO_4$, $SrCO_3$ etc. West Germany, Poland, Spain and USSR excluded. Increased demand expected
Ta	6 600	13 300	100.0 %	Including Nb
Tb				
Te	150	190	32.9 %	
Th	110	230	110.0 %	Import USA in 3 800 tons monazite as ThO_2

Table 1. (Cont'd).

Metal	1965 Production	1969 Production	Increase	Comment
Ti	2 500 000	3 200 000	30.6 %	Ilmenite
	220 000	380 000	69.4 %	Rutile
Tl		0.27		Import USA, unprocessed
Tm				
U	17 000	19 000	12.0 %	Czechoslovakia, East and West Germany, Hungary, India, and USSR excluded
V	8 900	10 300	15.5 %	
W	27 000	33 000	20.7 %	Scheelite
Y				
Yb				
Zn	4 300 000	5 300 000	23.7 %	
Zr	290 000	380 000	29.0 %	USSR, Eastern Europe and China excluded

Table 2. Uses of metals (From Bryan, 1977).

Metal	Uses of metals and compounds with importance generally decreasing from left to right. Percentages are for USA[a)]
Ag	Photography; electric conductors; sterling ware; solders; coinage; electroplating; catalysts; batteries.
Al	Building and construction; transportation; electrical industry; packaging; consumer durables; machinery; glass; pharmaceuticals.
As	Pb and Ca arsenate and sodium arsenite pesticides; glass and enamels; alloys; electronics.
Cd	Electroplating (50 %); pigments (22 %); thermoplastic stabilizers, e.g. in PVC (22 %); batteries; low melting point alloys.
Co	Alloys mainly; catalysts; pigments; enamels; glazes; electroplating.
Cr	Metallurgy-alloys (64 %); refractory bricks (21 %); electroplating; tanning; paint; wood preservatives.
Cu	Electrical industry; alloys, e.g. brass; chemical catalysts; antifouling paint; algicides; wood preservatives.
Fe	Iron and steel industry.
Hg	Chlor-alkali production; electrical apparatus; anti-mildew paint; instruments; catalyst; pesticides; pharmaceuticals; antifouling paint.

---

a) Information taken from Minerals Yearbook (1971).

Table 2. (Cont'd).

Metal	Uses of metals and compounds with importance generally decreasing from left to right. Percentages are for USA[a]
Mn	Metallurgy-largely steel alloys; dry batteries; chemical industry, e.g. permanganate; glass; ceramic coloring.
Mo	Metallurgy-steel alloys; catalysts; pigments; glass; lubricant and oil additive.
Ni	Metallurgy-steel and other alloys; electroplating; catalysts.
Pb	Storage batteries (42 %); leaded gasoline (19.5 %); pigments, e.g. red lead paint; ammunition; solders; cable covering; anti-fouling paint.
Sb	Antimonial lead (hardened lead); plastics; ceramics and glass; flame-proof chemicals; bearing metal; pigments; type metal.
Se	Glass industry; electrical industry, e.g. rectifiers, photocells; paints; rubber; insecticide-sodium selenite.
Sn	Tinplate; solders; bronze; white metal; chemical reducing agent; fungicide-triphenyl tin acetate; anti-fouling paint.
V	Metallurgy-steel and other alloys; catalyst; pigments.
Zn	Zn-based alloys; brass and bronze; galvanizing; rolled Zn; paints; batteries; rubber.

---

a) Information taken from Minerals Yearbook (1971).

Table 3. Estimated total emission of metals (metric tons/year) from oil combustion (from approximately 30 x 10^6 tons) in Sweden 1971 (From Andersson and Grennfelt, 1973).

As	0.7
Cd	0.2
Co	4.4
Cr	0.82
Cu	3.9
Fe	67
Hg	0.075
In	0.001
Mn	1
Mo	2.4
Ni	180
Pb	10
Sb	0.075
Sc	0.007
Se	2.5
V	580
Zn	10

Table 4. Transmethylation reactions attempted in aqueous solutions[a] (From Jewett and Brinkman, 1974).

Methylator	$HgCl_2$	$NaAuCl_4$	Na_2PdCl_4	K_2PtCl_4	$PtCl_2$[d]	Me_2TlCl	Me_2SnCl_2	$CdCl_2$
Me_3Sn^+	+[b]	+	+	+	0			
Me_3Pb^+	+[b]	+	+	+		0	0	0
$MeHg^+$		+	+	+			0	
Me_2Tl^+	0		+					
Me_2Sn^{++}	0		0					
Me_3S^+	0[c]							

a) Reactions were carried out in 5 mm (outer diameter) nuclear magnetic resonance tubes at ~10^{-2} M for each reactant. Plus signs indicate cases where reaction occurred and zeros indicate cases where no reaction was observed over a 100 hr period.

b) Rate of transmethylation retarded by presence of CN^- or SCN^-.

c) No reaction up to 100°C.

d) Conducted in degassed water.

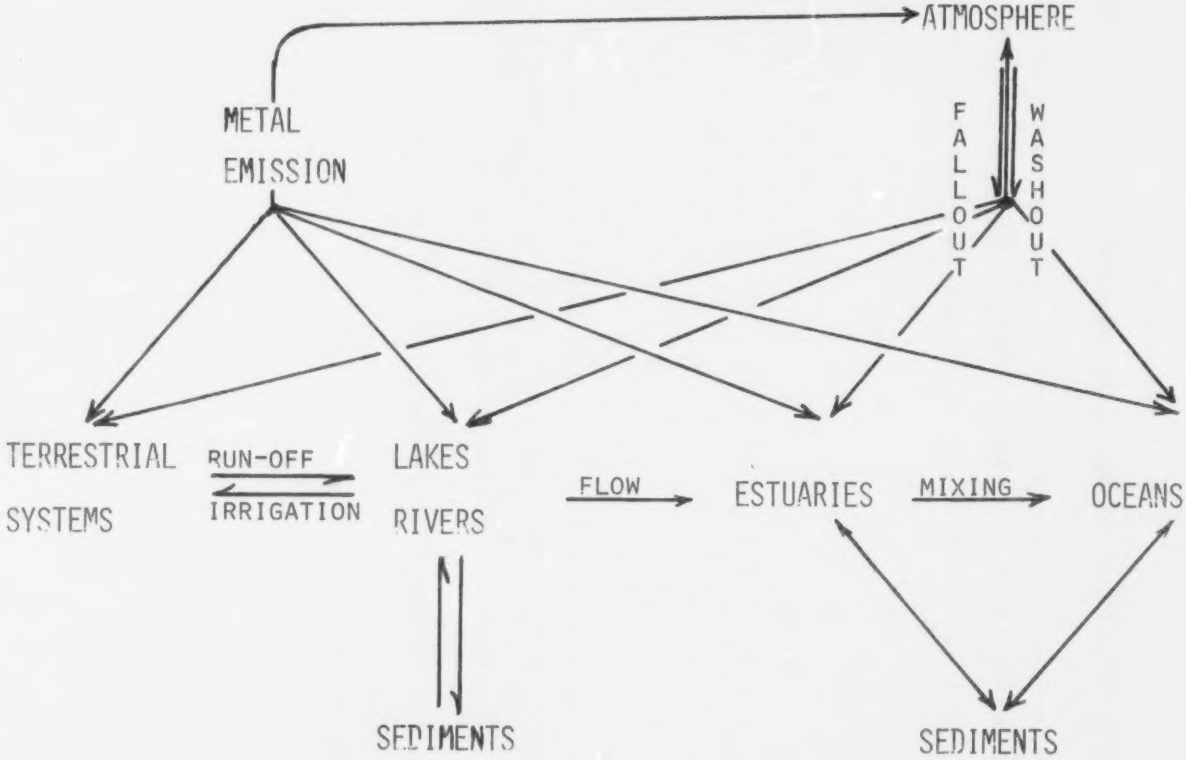

ATMOSPHERE
METAL EMISSION
FALLOUT
WASHOUT
TERRESTRIAL SYSTEMS
RUN-OFF
IRRIGATION
LAKES RIVERS
FLOW
ESTUARIES
MIXING
OCEANS
SEDIMENTS
SEDIMENTS

Figure 1. Routes for trace elements in the environment.

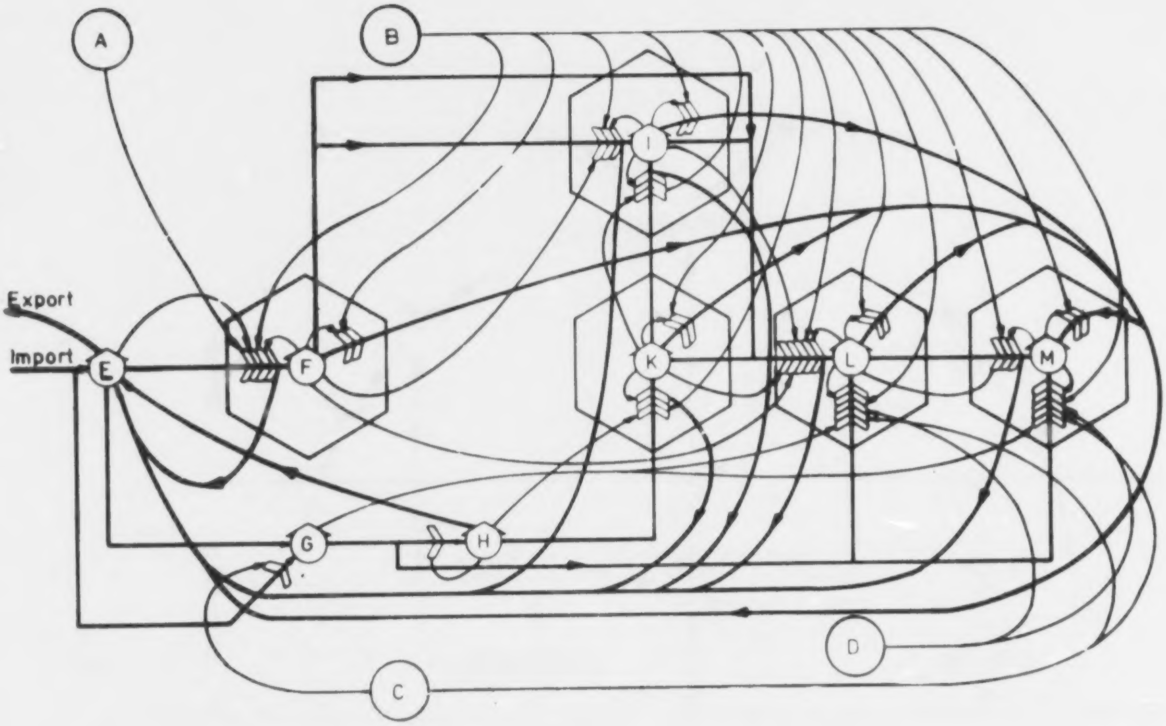
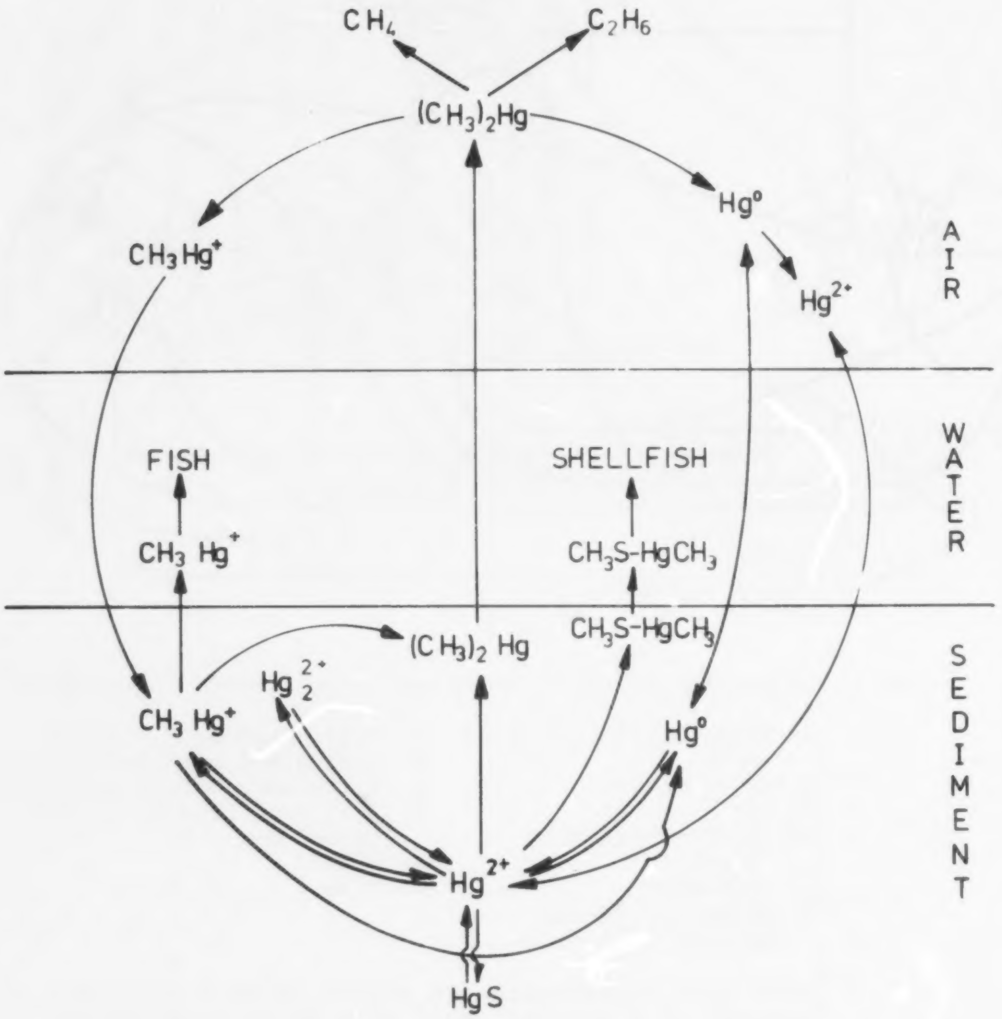


Figure 2. Mercury transport in a simplified limnic food chain.

A. Composition of sediment	G. Methylmercury in water
B. Temperature	H. Suspended organic material
C. pH	I. Predatory bottom organisms
D. Concentration of oxygen	K. Detritus feeding bottom organisms
E. Sediment	L. Roach
F. Sediment feeding bottom organisms	M. Pike

Each "box" represents the amount of methylmercury present in a trophic level at a given time. Boxes surrounded with hexagons indicate living organism populations.

The broad arrows represent flow of mercury and the narrow ones flow of information. The sources of information can be either "outside the system", indicated by circles, which means that they have an influence on the system without being influenced by it, or "inside the system", indicated by roofed circles, which means that they affect and are affected by the system itself; feedback mechanisms.



CH_4
C_2H_6
$(CH_3)_2Hg$
Hg^0
CH_3Hg^+
Hg^{2+}
AIR
FISH
SHELLFISH
CH_3Hg^+
$CH_3S\text{-}HgCH_3$
WATER
$CH_3S\text{-}HgCH_3$
$(CH_3)_2Hg$
Hg_2^{2+}
CH_3Hg^+
Hg^0
SEDIMENT
Hg^{2+}
HgS

Figure 3. The mercury cycle.

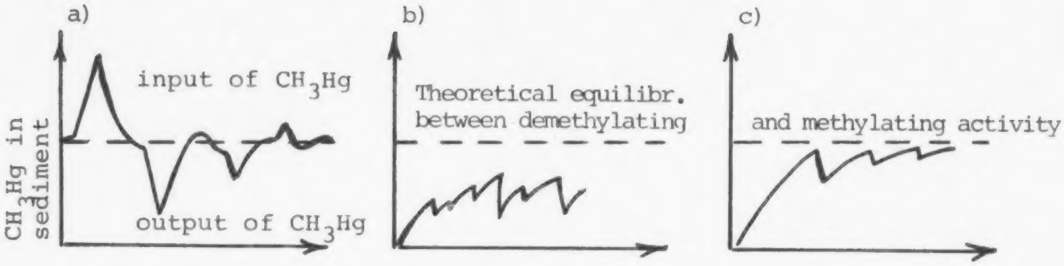

a)
input of CH3Hg
CH3Hg in sediment
output of CH3Hg
b)
Theoretical equilibr. between demethylating
c)
and methylating activity

Figure 4. The combined effect of input and output, methylation and demethylation of mercury.

EFFECTS - GENERAL PRINCIPLES UNDERLYING THE TOXIC ACTION OF METALS

Thomas W. Clarkson

Metals produce a wide range of effects in man resulting from their action at molecular, cellular, tissue and organ levels. Depending upon the particular metal involved, the metal action may manifest itself as a local effect such as on the skin, pulmonary membranes or gastrointestinal tract or it may manifest itself as a systemic effect that could potentially involve any tissue or organ in the body. Furthermore, metals may act as allergens, mutagens, teratogens or carcinogens. No group or class of effects is unique to the metals. Indeed it is the diversity of effects that, if anything, is characteristic of the metals.

A comprehensive, all-inclusive description of the biological effects of metals would amount to a textbook of general pathology. Instead this chapter will attempt to discuss the general principles underlying the toxic action of metals. Effects associated with a particular metal are described in the chapters of this book dealing with specific metals.

1. Metals as elements

1.1 Cumulative action of metals

Unlike the majority of organic chemicals that can be eliminated from tissues by metabolic degradation the metals as elements are indestructable and therefore have the potential for accumulation in the body leading to chronic effects. The only way metals can be eliminated from the body is by excretion. The rate and pathways of excretion vary greatly from one metal to another. For example, the biological half-time of methylmercury in man is about seventy days versus an estimated half-time of ten to twenty years for cadmium. The biological half-time for one and the same metal may also vary between different tissues. The biological half-time of lead in tissues is in the order of a few weeks versus an estimated biological half-time of about ten years in bone.

Accumulation in tissues does not necessarily imply the advent of toxic effects. In the case of certain metals inactive complexes or storage depots are formed. Lead is stored in bone normally in an inert form. Only on the release from bone that may occur under certain physiological conditions will lead poisoning result. As will be discussed in detail elsewhere in this book cadmium and other metals bind to a small molecular weight protein, metallothionein, that appears to form inert complexes. Only when such complexes reach a critical concentration in the cell do toxic effects appear. Inorganic mercury, cadmium and other metals may form inert complexes with selenium compounds. The formation of such complexes results usually in the retention of the metal in body tissues. The long-term sequelae of storage of these complexes over a human lifespan is not yet fully understood. It is clear from animal experiments that the formation of these complexes can prevent the acute and short-term toxic effects of these metals. However, it is not clear to what extent storage of these complexes over long periods of time is without hazard. For example, cadmium is believed to accumulate in human kidney tissue over virtually the entire lifespan of the individual. In members of the normal population such an accumulation of cadmium can attain values of up to 50 mg/kg. The estimated critical concentration for effects is around 200 mg/kg. This long-term accumulation of cadmium is a matter of concern given that levels in later life are approaching those associated with tissue damage.

1.2 Metals as essential and non-essential trace elements

The presence of many of the metals in the earth's crust can no doubt be interpreted as showing that they are essential for life processes. Metals are usually essential because they are an integral part of at least one enzyme. Since the primary action of an essential metal is as catalysts only trace amounts of the metals are necessary for optimal cellular function.

The dose-response relationships for the essential metals therefore must take into account the fact that at very low intakes of the metal biological effects may appear due to deficiencies whereas at high intakes effects may be due to an overdosage.

The consequences of deficiency states of essential trace metals are described in great detail in a variety of nutritional textbooks. In general, deficiency results in retarded growth along with specific effects associated with each metal. In certain cases the deficiency state may not be associated with reduced amounts of metal in the diet. Zinc deficiency may be caused by reduced reabsorption of the metal from the diet and also by elimination of the metal in perspiration. Deficiency states may be produced by competitive action of other metals as shall be discussed below. Thus the fact that certain trace elements are essential to life leads to the possibility of interaction with other metals in producing biological effects.

The body has developed a variety of homeostatic mechanisms with regard to the essential trace metals. These mechanisms usually take the form of a controlled absorption from the diet allowing enhanced absorption with low dietary intakes and diminished absorption with high dietary intakes. One of the most studied is that of iron whereby at low intakes or in the face of major biochemical demands for this element, absorption across the intestinal barrier is increased. When body stores are adequate, iron is stored in the mucosal cells as ferritin and is eliminated from the body when the intestinal cells exfoliate. Specialized carrier proteins have been identified for the essential trace metals such as transferrin which carries in plasma such metals as iron, cobalt and zinc. A calcium transport protein has been identified in kidney tissue and in mitochondrial membranes. Non-essential metals may participate in some of these specialized processes, resulting in toxic effects directly or by interaction with the essential trace metals.

The essential trace metals are less likely to produce toxic effects because of the presence of homeostatic mechanisms that control the body burden. Nevertheless there are a variety of ways in which the control mechanisms have been overwhelmed or circumvented. The actions of metals on skin or pulmonary membranes are independent of homeostatic controls. For example when the trace metal is present as a corrosive or acid forming salt, effects on skin and the mucosal membranes may appear. Although chromium is an essential trace element, chromate salts can produce severe effects in both the skin, nasal septum and in the lung. The homeostatic mechanisms usually operate to control gastrointestinal absorption so that entry into the body by other pathways circumvents this control. For example, the inhalation of zinc oxide fumes can give rise to an allergic response resulting in metal fume fever. Inhalation of manganese oxides or salts can result in chronic toxicity. The homeostatic control can be overwhelmed by very high doses of the essential metal such as is the case with iron salts. Above a certain dosage, destruction of the gastrointestinal tract is so severe that the iron enters the bloodstream in toxic quantities.

The toxic effects may take forms which are not clearly unique for the essential metals except in the case where the action of the enzyme involving the metal is enhanced. For example excessive doses of molybdenum may increase the activity of the molybdenum-requiring enzyme xanthine oxidase resulting in the increased production of uric acid. As discussed in the chapter on molybdenum, this may account for reports of gout-like symptoms in people having excessive exposure to this metal.

Some of the toxic effects of the non-essential trace metals may be a consequence of a blockage of the availability or activity of essential metals. Xanthine oxidase is inhibited by tungstate salts due to the displacement of molybdenum. Lead blocks the utilization of iron in heme synthesis by inhibition of the enzyme ferro chelatase. There is evidence that cadmium can block the entry of zinc into the fetus thereby causing a variety of teratogenic effects.

The effect of lead in mitochondria may be a direct consequence of its carriage into the sub-cellular particle by the cadmium transport system. In fact the calcium carrier is believed to be relatively non-specific for calcium and capable of transporting a broad spectrum of divalent cations.

Competition effects probably occur between essential metals themselves. The toxicity of copper is attenuated by that of molybdenum and vice versa. Other examples of competitive interaction between specific metals can be found in the chapters on specific metals and in the chapter on factors affecting dose-effects and dose-response relationships.

2. Oxidation-reduction reactions of metals

All metals can undergo conversion from their atomic metallic form to a corresponding cation by the loss of one of more electrons. The opposite process (reduction) may also occur in tissues in a limited number of cases. Each metal has its characteristic oxidation-reduction or redox potential.

The toxicity of each metal depends both quantitatively and qualitatively on its oxidation state. These oxidation changes may be brought about either by chemical action, depending upon the local redox potential in the cell or biological fluid, or by enzymic action. In general the removal or addition of electrons to the metal atom will influence the chemical activity and therefore the potential of the metal to interact with tissue ligands as will be discussed in sections below.

Examples where the toxicity of certain metals is greatly influenced by the oxidation state are given in the relevant metals chapters but one illustration, mercury, will be given here as well. Mercury may exist in three oxidation states as illustrated in the general equation:

$$Hg^0 \rightleftarrows Hg_2^{++} \rightleftarrows Hg^{++}$$

Metallic mercury (Hg^0) may be oxidized to mercurous mercury (Hg_2^{++}) which may in turn undergo oxidation to mercuric mercury (Hg^{++}). The toxicity of these three oxidation states of mercury differs considerably. Metallic mercury gives rise to symptoms and signs in man associated almost exclusively with damage to the central nervous system. Mercurous mercury forms a limited number of salts. The most common of these is mercurous chloride or calomel which is highly insoluble. Its toxic properties are mainly that of an antiseptic or local irritant probably due to the release of a small amount of ionized mercury. The divalent form of mercury forms a variety of inorganic mercuric salts most of which have a high acute toxicity. The signs and symptoms resulting from these forms of mercury relate in part to its local action as a corrosive chemical, such as damage to the gastrointestinal tract and in part to systemic action, mainly kidney damage. Hemorrhage in the lower gastrointestinal tract is also a possible systemic consequence of ingestion of inorganic salts of mercury. The reason for the qualitative differences in toxicity between metallic mercury and inorganic mercury salts is not fully understood but studies in recent years indicate the importance of oxidation reactions in explaining some of these.

Mercury vapor inhaled into the pulmonary spaces of the lung is believed to cross the alveolar membranes rapidly due to its high diffusibility (mercury vapor is a monatomic gas) and high lipid solubility (the heptane to water ratio is about 20). On entering the plasma where it dissolves in the metallic atomic form it rapidly diffuses into the red blood cells to undergo oxidation to divalent ionic mercury. The latter binds to ligands in the red cells (Figure 1). However, a certain amount of the dissolved vapor persists in the bloodstream for a sufficient length of time (about 15 seconds) to reach the blood-brain barrier which it easily crosses. Once in the brain tissue it undergoes oxidation to ionic mercury and again is available for binding to tissue ligands. Inorganic mercury in the ionic form is not available for transport across the blood-brain barrier in substantial amounts because it is present in plasma in a protein-bound form. In this metabolic scheme it is clear that the rate of oxidation of the inhaled vapor is a critical process controlling the amount of mercury available for diffusion across the blood-brain barrier.

A considerable amount of work has now been carried out on the mechanisms of the oxidation step. Work by Stock (1934) showing that mercury vapor was oxidized to mercuric oxide in aqueous solutions in the presence of oxygen and work by Clarkson et al. (1961) showing that oxygen accelerated the in vitro uptake of mercury vapor by blood suggested that a purely chemical reaction was involved. However, the finding that uptake by red cells was much more rapid that that by plasma suggested the involvement of metabolic factors. This was confirmed by the findings of Nielsen-Kudsk (1965) that ethanol depressed the retention of mercury vapor by man and that ethanol could depress mercury vapor uptake in vitro by blood. He also demonstrated that the generation of hydrogen peroxide inside the red cells was an important factor in the oxidation of mercury vapor. Subsequent studies have pointed to the hydrogen peroxide catalase system as the primary pathway of oxidation of mercury vapor not only in red cells but in liver and probably in other tissues (Magos et al., 1977).

The oxidation action has important implications for the metabolism of inhaled mercury vapor in the body and therefore for the determination of health effects. Not only will the oxidation reaction determine the residence time of the inhaled vapor in the bloodstream and therefore its potential of reaching such sensitive sites as the central nervous system and the fetus but it also will allow the possibility of interaction with other chemicals or genetic states which affect the activity of catalase. The interaction between alcohol and mercury vapor has been mentioned. Population groups which are genetically deficient in catalase have been described in several countries. Even in the normal population, catalase activity in red cells has a wide range of values.

That the hydrogen peroxide catalase system is responsible for the oxidation of mercury vapor has important implications with regard to the mechanism of action at the sub-cellular level. It would seem likely that the ionic species is produced within the cell wherever sufficient amounts of hydrogen peroxide and catalase occur. In many cells this could be the peroxisomes and lysosomes. Norseth and Brendeford (1971) have pointed to the possibility that lysosomes damaged by mercury may release hydrolytic enzymes. This could be the primary mechanism of injury to the cell. The evidence that mercury vapor is probably oxidized to ionic mercury within the peroxisome would lend further support to Norseth and Brendeford's ideas.

3. Organometallic compounds

Many metals are capable of forming covalent bonds with the carbon atom to create organometallic compounds. In general the toxic properties of these compounds differ drastically from those of the inorganic forms of these metals. Tetraethyl lead, triethyl tin, trimethyl bismuth and methylmercury, in contrast to the inorganic forms, can produce severe damage to the central nervous system. Inorganic lead does give rise to nervous effects but they are different from those produced by the organic form of lead. In general the toxicity of the longer chain alkyl derivatives of these metals is less than that of the short chain compounds. Their toxicity to the central nervous system is undoubtedly associated with their ability to penetrate the blood-brain barrier rapidly.

The alkylation of lead, tin and bismuth does not occur in mammalian tissues. In the case of tellurium and trivalent arsenic, methyl derivatives are formed in the body but these appear to be relatively non-toxic. Dimethyl telluride is rapidly exhaled from the body and dimethylarsonic acid and dimethylarsinic acid appear to have a low toxicity.

Cleavage of the metal carbon bond occurs in the body in the case of many of these organometallic compounds. In the case of tetraethyl lead, the trialkyl metabolite is the toxic species. In other cases, such as that of the carbon mercury bond, cleavage may serve as a detoxification pathway. The role of biotransformation in the toxicity of organometallic compounds has been most extensively studied and is best illustrated in the case of mercury, which forms a wide variety of organomercuric compounds. A brief discussion will be given below of three classes of carbon mercury bonds in terms of the way in which the stability of the bond determines the toxic properties of these compounds.

The short chain alkyl mercurials (ethyl, methyl and propyl mercury) have probably the most stable mercury bonds of all the organomercurial compounds. The stability of the carbon mercury bond in methylmercury compounds is illustrated in Figure 2. This records the blood concentration of methylmercury upon hospital admission and during the subsequent period of almost one year in a person who had been exposed to methylmercury in an outbreak in Iraq. It may be seen that although the methylmercury concentrations in blood eventually declined to background values, the organic form of mercury persisted in this patient for at least one year. Inorganic mercury levels were also elevated at the time of exposure and shortly afterwards. This was probably due to a slow conversion of methyl to inorganic mercury. However, to explain the persistence of methylmercury in the body for one year this conversion must be less than 1% per day.

Conversion to inorganic mercury in the body probably does not play a direct role in determining the damage inflicted on the central nervous system in man or animal. At one time it was suspected that the latency period between the end of exposure and the appearance of signs or symptoms might be explained by a slow accumulation in the brain of inorganic mercury split off from the carbon atom. However, studies in animals indicate that no such accumulation of inorganic mercury takes place. The opposite is true for the liver and kidney where there is evidence from animal experiments to indicate that effects on these organs may be due to inorganic mercury split off from the parent molecule. The accumulation of inorganic mercury in kidneys of rats given methyl- and ethylmercury compounds is shown in Figure 3. After repeated doses the increase in the concentration of inorganic mercury in the kidneys of these animals is substantial. The kidney level associated with acute damage following a single dose of mercuric chloride is usually between 20 and 40 mg/kg wet weight. It may be seen that on chronic dosing with methyl- or phenylmercury compounds the level of inorganic mercury can far exceed levels associated with acute damage. This high level of inorganic mercury probably causes the ultra-structural and biochemical changes seen in rats. Even so, no gross structural damage is observed. These observations suggest that the inorganic mercury released or accumulated in the kidney slowly over a period of time due to the cleavage of the carbon mercury bond is better tolerated by the tissue than a single acute dose. The inorganic mercury is perhaps able to stimulate the synthesis of metallothionein in this tissue and it might then serve in a protective role. Studies of these possibilities have not been reported.

Although the role of conversion to inorganic mercury in determining the toxic effects of methylmercury seems to be minimal, it is important in excretion of methylmercury from the body. Most of the elimination of mercury from the body in animals or humans given methylmercury takes place via the feces. Inorganic mercury is at least 50% of the total mercury in the feces of experimental animals and in humans it may amount to 100% (Turner et al., 1975). These data suggest that conversion to inorganic mercury may be the rate-determining step in the excretion of methylmercury from the body. Where this conversion to inorganic mercury is occurring is not satisfactorily established. In animals given methylmercury some inorganic mercury is excreted in the bile but not enough to explain the fecal excretion of inorganic mercury. It has been suggested (Norseth and Clarkson, 1971) that microflora in the intestine might be able to convert methylmercury to the inorganic form and thereby contribute to fecal excretion. This methylmercury might originate as methylmercury secreted from the bile or as methylmercury exfoliated from intestinal cells. Some preliminary experiments indicated that microflora in vitro were able to convert methyl to inorganic mercury. The situation is not completely clear since further experiments by Norseth (1971) indicated no difference in excretion rate or biological half-time of methylmercury in germ-free animals as compared to normal animals. This is an area of considerable research interest as we are in great need of understanding the mechanisms whereby this hazardous form of mercury is eliminated from the body as well as the very large individual variations in the biological half-time of methylmercury in man.

The aryl and alkoxyalkyl mercurials form another distinct class of organomercurials that are less toxic than the alkyl compounds. One reason for their low toxicity is their rapid conversion to inorganic mercury. There is evidence that the conversion takes place in the liver and is mediated by an enzyme in the cytosol of the liver cell. The mechanism is not understood in detail except that it is believed to occur by reductive cleavage of the carbon mercury bond.

The organomercurial diuretics form an important class of mercury compounds. Considerable investigations have been carried out as to their biological action and the mechanisms thereof. Despite the introduction of a variety of new types of diuretic agents in recent years, the organomercurial diuretics still provide one of the most selective ways in which to inhibit active sodium transport in the renal tubule. This in itself is surprising insomuch as organic mercury cations can react with a variety of tissue ligands and would therefore not be expected to be so selective in their biological action. Investigations have been carried out to try to discover how compounds that are so unselective in their actions in vitro could produce such selective and graded damage in vivo.

An important landmark in these investigations was the publication of the mercuric ion hypothesis by Weiner et al. (1962). The assumptions of this hypothesis are depicted diagrammatically in Figure 4. The organomercurial diuretic is present in plasma bound mainly to plasma proteins but a small fraction is bound to a diffusible amino acid presumed to be cysteine. The diffusible and non-diffusible compounds are in equilibrium. The cysteine-bound form may enter the tubular fluid via filtration through the glomerulus and via excretion across the tubular cells. The organomercurial is reabsorbed in the lower proximal tubule. At this point the mercurial compound may bind to non-specific sites in the cell, resulting in no biological effects. Alternatively the organomercury compound may undergo cleavage of the carbon mercury bond releasing ionic inorganic mercury to bind to a specific receptor site leading to inhibition of sodium transport. The receptor site is bidentate. The divalent inorganic mercury forms a ring structure with the two ligands. Formation of this ring is the essential prerequisite for diuretic action.

This hypothesis has some support by virtue of the fact that in vitro all organomercurial diuretics are unstable and readily release inorganic mercury under acid conditions in the presence of a complexing agent such as cysteine. The mercuric ion hypothesis also offered a simple explanation for the well-known fact that mercurial diuresis is potentiated by acidosis as well as for the fact that certain organic mercury compounds such as parachloromercury benzoate and paramercuribenzene sulphonate are able to block the diuretic action of organomercurials. Both of these compounds proved to have stable carbon mercury bonds in the in vitro test. It was argued that they would attach to only one ligand in the receptor and thereby either displace inorganic mercury from the ring structure or prevent inorganic mercury from forming the ring structure with the receptor (Millar and Farah, 1962). Indirect support was given to the mercuric ion hypothesis by the observation that mercurial diuretics do liberate inorganic mercury in vivo in kidneys of rats and dogs. At the same time, it was observed that non-mercurial diuretics also released inorganic mercury and that the levels of inorganic mercury in the kidney tissue were not increased during acidosis (Vostal and Clarkson, 1973). At the present moment therefore the mercuric ion hypothesis cannot be said to be definitively established but it has many attractive aspects in the context of trying to understand the selective toxicity of metals at the molecular level.

The formation of chelate ring structures by polyvalent metals may play a role in their selective toxicity. An example is the case of trivalent arsenic which forms a ring compound with lypoic acid, resulting in the inhibition of keto acid dehydrogenases. This is believed to be the biochemical lesion behind the biological effects.

4. Reaction of metals with ligands

Metal cations may form coordinate-covalent bonds with a wide variety of electron donating groups (ligands). The overall reaction is represented as:

$$M + L \rightleftharpoons ML$$

where M is the metal cation and L is the monodentate ligand. The equilibrium constant or affinity constant (K) is expressed by the Mass law relationship

$$K = \frac{[ML]}{[M].[L]}$$

Most ligands of biological importance contain the oxygen, nitrogen or sulfur atom.

The biological consequences of metal interaction with ligands have recently been discussed in detail by Webb (1977). Some of the immediate ones are breaking of hydrogen bonds, displacement of other metals from a ligand site and change in the tertiary structure of a protein leading to inhibition of catalytic activity. The attachment of metals to ligands in membrane structures can lead to inhibition of active transport or to alteration in the passive permeability properties of the membrane. Interaction of metals with ligands of nucleic acids potentially could affect both transcriptional and translation processes and may underlie the mutagenic and carcinogenic action of certain metals. Inhibition of DNA repair enzymes might also contribute to the mutagenic and carcinogenic potential of a metal.

Each metal possesses its own spectra of affinity constants for different tissue ligands. These are illustrated for methylmercury and mercuric ion cations in Table 1. The wide range of values for the affinity constants expressed in logarithmic units is obvious. Significantly high affinity constants are seen even with such simple ligands as hydroxyl and chloride anions. In the case of methylmercury, the highest values are seen in molecules containing the sulfhydryl group. Other metal cations will usually exhibit a different spectra of affinity constants. Nevertheless, it appears to be a general and implicative rule that most metal cations can form stable complexes with a wide variety of ligands. This raises the question as to how metals that are so reactive to such a wide variety of tissue ligands can produce such selective actions in the whole animal. This question is by no means fully answered at this time but this section will try to deal with the general principles of metal interaction with ligands and to describe a few cases where selective actions of a metal may be understood.

4.1 Interaction of metals with diffusible ligands

Following absorption from the gastrointestinal tract or the lung the metal may enter the bloodstream as the free cation (M) or in a complexed form (ML) and may undergo reactions with diffusible ligands in plasma (L^1) as follows:

$$M + L^1 \rightleftharpoons ML^1$$

or $$ML + L^1 \rightleftharpoons ML^1 + L$$

Examples are known where these ligands modify the toxicity of the metal and play a decisive role in determining the site of action in the body.

The beryllium cation (Be^{++}) has a high affinity for oxygen-containing ligands such as hydroxyl and phosphate. Immediately upon entering the plasma, beryllium is believed to form insoluble compounds involving hydroxyl and phosphate anions, leading to the formation of colloidal beryllium salts. This accounts for the observation that beryllium is rapidly taken up by the reticulo-endothelial system following its absorption into the bloodstream. Subsequent removal of beryllium from the RE cells in the liver and its transport to bone is believed to be the result of a slow solubilization of the beryllium cation. The carrier molecule in this case is unknown.

Ionic lead rapidly forms insoluble precipitates with inorganic phosphate anions. The reaction is highly pH dependent and involves the interaction of lead with the trivalent phosphate anion (PO_4^{3-}). Early studies by Aub and his co-workers (1926) clearly identified the role of phosphate in limiting the solubility of lead in plasma. Lead phosphate colloids will carry a net negative charge in the presence of excess phosphate in plasma. These colloids have the potential for absorbing organic molecules such as amino acids or proteins to form a highly dispersed colloid in the form of peptised sol. It has been proposed that the initial step in lead uptake by red cells involves the adsorption of the small colloidal particles on the surface of the red cell. There is some electron microscopical evidence to support this pathway of uptake (for review see Passow et al., 1961). Eventually lead may penetrate into the interior of the cell where it is available to interact with internal cellular ligands, resulting in the inhibition of the enzyme ALA-dehydratase. Lead may also enter the red cells during hematopoiesis. At the present moment it is not clear what fraction of the lead in the interior of the cell originates from lead uptake during development of the cell and what fraction represents lead penetration across the red cell membrane.

The interaction of the uranyl ion (UO_2^{++}) with bicarbonate anions and the carboxylate groups of serum proteins was studied intensively in the 1940's and became a classic example of the importance of metal ligand interaction in determining the selective action of a metal cation. It was found that uranium absorbed into the bloodstream as UO_2^{++} rapidly formed complexes with bicarbonate and with carboxylate anions on serum protein. The bicarbonate complexes were readily filterable in the kidney and entered the proximal tubular fluid. Acidification processes in the proximal tubule removed the bicarbonate anions, liberating the free uranyl cation. Thus UO_2^{++} became available for reaction with cellular ligands in the proximal tubule of the kidney. This mechanism is believed to be the explanation for the fact that UO_2^{++} damages primarily the cells of the proximal tubule. Moreover such a mechanism gave an explanation for the dramatic effects of acidosis and alkalosis on the excretion and toxicity of uranyl salts. In acidotic animals the bicarbonate removal from urine is complete so that most of the uranium filtered as the bicarbonate complex is deposited into the renal cortex. On the other hand in animals made alkalotic by treatment with sodium bicarbonate a substantial amount of the bicarbonate passes into the urine, carrying with it an appropriate quantity of UO_2^{++}. Alkalotic animals thus have considerably less deposition in the kidney tubules and show fewer toxic effects of uranium.

The interaction of metals with organic diffusible molecules such as amino acids is less well-documented in vivo. Most metals are either protein-bound or attached to the red cells in blood so that the amount actually in a diffusible form is quite small.

The identification of the chemical nature of these diffusible complexes therefore presents considerable analytical problems while at the same time these complexes may be quite important in terms of the toxicity of the metal, as has been well demonstrated in in vitro studies.

Vander (1962) has shown that the infusion of a cadmium cysteine complex into the arterial circulation of the kidney in dogs leads to an enhanced reabsorption of sodium in the proximal tubule. This might be important for some of the cardiovascular effects of cadmium. In vitro studies on the isolated intestine indicated that silver complexes of cysteine also stimulate active ion transport (Clarkson and Toole, 1964). It is not clear from the available data whether the cysteine complex itself interacts with the membrane to produce these transport changes or whether the cysteine modifies the chemical potential of the metal, allowing only small amounts of the free metal cation to react with the membrane sites.

One matter that is clear is that the cysteine complexes of certain metals differ from the inorganic salts in terms of binding activity. For example, the chloride salts of inorganic mercury and a variety of organomercurial diuretics are potent membrane poisons that can both inhibit active transport and increase membrane permeability. An attempt has been made to explain the selective localization of the effects of organomercurial diuretics in the renal tubule in terms of a supposed equilibrium between protein amino acid and chloride complexes of these mercurial compounds (Clarkson and Vostal, 1973). It is assumed that the mercurial diuretic enters the tubular fluid either by filtration at the glomerulus or by active transport across the tubular cells. Following similar arguments as were used for bicarbonate complexes of uranium, it is then assumed that the organomercurial cysteine complex undergoes dissociation as cysteine is reabsorbed in the proximal tubules of the kidney. This allows the formation of the chloride complexes of the organomercurials since the chloride concentration is maintained, if not increased, as the tubular fluid moves along the nephrons.

The reactions occurring within the renal tubular fluid were assumed to be:

$$\mathrm{R\text{-}Hg^{+} + Cl^{-} \rightleftharpoons R\text{-}HgCl}$$

$$\mathrm{R\text{-}Hg^{+} + Cys^{-} \rightleftharpoons R\text{-}HgCys}$$

$$\mathrm{Cys^{-} + H^{+} \rightleftharpoons CysH}$$

where $\mathrm{R\text{-}Hg^{+}}$ is the organomercurial cation and CysH and $\mathrm{Cys^{-}}$ are the cysteine molecules with undissociated and ionized sulfhydryl groups, respectively.

It may be seen that increasing the acidity as well as increasing the chloride concentration will facilitate the formation of the chloride complex as the amino acid is reabsorbed. Making reasonable assumptions concerning the affinity constants of the organomercurial for the sulfhydryl group and for the chloride anion and the pK of the sulfhydryl group, it is possible to estimate that a small fraction of the mercurial would indeed be present as the chloride complex in the renal tubular fluid. This may be the species that penetrates into the cell where it may undergo biotransformation, allowing inorganic mercury to react with the bidentate receptor as discussed previously.

The formation of amino acid complexes with metals may in some cases be enzyme-mediated. Methylmercury is excreted in the bile of mice and rats as a small molecular complex with a sulfhydryl compound, which may be the amino acid glutathione. The process may be catalyzed by conjugation enzymes in the liver such as glutathione transferase, although evidence on this is still not conclusive. Such complexes secreted into the bile undergo reabsorption in the gastrointestinal tract. This is known to be true for methylmercury and possibly for other cations. Such small molecular weight complexes, formed within the liver, secreted in bile and reabsorbed into the bloodstream may represent an important mobile form of the metal in the body, allowing the metal to reach its site of action (Norseth, 1973).

4.2 Interaction of metals with macromolecules

The interaction of metals with nucleic acids and proteins has been studied extensively in vitro. Unfortunately in most of these types of experiments, the conditions were unphysiological. The presence of small diffusible anions such as chloride, bicarbonate or amino acids could substantially change the interaction with ligands and macromolecules. In most cases it is impossible to extrapolate in vitro physiochemical observations to the situation in the whole animal.

For theoretical reasons it is clear that interactions with macromolecules are probably responsible for the toxic effects of most metals. The receptor for the toxic action is probably in most cases a macromolecule functioning catalytically or as a structural or transport component of a membrane.

Webb (1977) has discussed the interaction of heavy metals with nucleic acids and has pointed to phosphate groups and nitrogen atoms in heterocyclic bases as likely sites for coordinate covalent bonding with metal cations. Such metals as cadmium and copper probably bind to the heterocyclic bases, resulting in the breaking of hydrogen bonds and the destabilizing of the DNA structure. Metals may also be essential for enzymes that are involved in the synthesis and repair of nucleic acids. Zinc is necessary for the catalytic activity of thymidine kinase. Its replacement by other metals such as cadmium may affect the functioning of this enzyme and may therefore have effects on DNA synthesis. The mutagenic activity of certain arsenic compounds has recently been attributed to the inhibition of DNA repair enzymes by this metal (Rossman et al., 1977).

The interaction of metals with proteins and particularly with enzymes has been subjected to more extensive study than any other area of metal interaction on the molecular level. Many factors influence this interaction, as demonstrated by a variety of in vitro studies. For example, the pH of the medium is important since hydrogen ions compete with the metals for binding to most of the ligands. Diffusible anions such as chloride and hydroxyl will limit the reactivity of the metal cation with the macromolecular ligand. Some of the ligands in the macromolecule may be buried within the tertiary structure and not readily available for interaction with the metal cation. Reaction of the metal cation with external ligands on the protein may lead to the unfolding of the protein chains, leading to time-dependent reactions. Neighboring groups may influence the reactivity of the metal with a particular ligand. These factors considerably influence the type of inhibition that metals can exert on an enzyme. Direct interaction of the metal with the active site follows the kinetics of competitive inhibition. For example, mercury inhibits the enzyme invertase but does so competitively with the substrate sucrose. A metal may react with sites adjacent to the active site of the enzyme, resulting in partial and non-competitive inhibition. The sites remote from the active center may also interact with the metal, which may produce either no effect or, in certain cases, quite dramatic effects possibly due to changes in the tertiary protein structure. An example of such a reaction is that between inorganic mercury and hemogloblin. Riggs (1952) has shown that although mercury does not react with the active oxygen binding sites in hemogloblin it does substantially change the oxygen dissociation curve of this molecule by changing the tertiary structure.

An interesting example of the selective action at the molecular level is the case of ionic inorganic mercury (Hg^{++}) and methylmercury (CH_3Hg^+) interaction with the receptor for acetyl chlorine. Shamoo et al. (1976) have contrasted the considerably greater ability of the CH_3Hg^+ as compared to Hg^{++} to block the activity of the isolated acetyl choline receptor. The proposed explanation is that CH_3Hg^+ because of its higher lipid solubility can penetrate regions of the acetyl choline receptor not available to Hg^{++}. Such a difference in ionic action may help to explain the findings that neuromuscular blockage can be produced by methylmercury in phrenic nerve diaphragm preparations and that muscular weakness can occur in patients poisoned by methylmercury but not in those poisoned by inorganic mercury (Rustam et al., 1975).

Some examples of enzyme inhibition in vivo by metals are well documented. The effect of lead on heme synthesis is well-known. The enzyme delta amino levulinic acid dehydratase (ALA/D) probably is the most sensitive enzyme with regard to lead. Its inhibition leads to decreased synthesis and to negative feedback control, which may in turn lead to the derepression of the enzyme delta amino levulinic acid synthetase and thus increase synthesis of delta amino levulinic acid. This appears in the urine as an early warning of the toxic effects of lead.

The inhibition of an enzyme may not always be the critical step for the biological or toxic effects of the metal for the whole animal. A classic example is the inhibition of the enzyme ALA dehydratase in red blood cells, which apparently plays little role in the biological effects of lead. The enzyme may have been important for the early development of the red cell but ceases to perform any useful physiological function in the mature erythrocyte.

The availability of macromolecular binding sites can be modified by the diet under certain circumstances and this may in turn affect the toxicity of the metal. Surtshin (1957) demonstrated that the sucrose feeding protected rats against the renal toxic effects of mercurial salts. It was subsequently shown that such a diet increased the protein bound SH in kidney. The protective effect probably could be attributed to the binding of mercury to those non-specific sites (Surtshin and Yagi, 1958).

Reaction with proteins is a necessary step in the antigenic action of metals. The pulmonary tissue reaction to beryllium is believed to be produced by cell-mediated immunity, probably involving protein complexes of beryllium. Metal fume fever seen after inhalation of zinc is also believed to be a result of reaction to antigenic protein complexes of zinc.

4.3 Interaction of metals with membrane-bound ligands

Rothstein (1973) has pointed out that even at toxic doses of a metal only a small fraction of tissue ligands is involved in heavy metal complexes. For example, assuming that methylmercury is attached to sulfhydryl groups in the red cells, a lethal concentration of 5000 μg/kg would involve the binding of only one sulfhydryl group in a thousand. Thus the important point to bear in mind is not the average properties of all the sulfhydryl groups but the properties of those few sulfhydryls that are involved in metal binding. The same principle applies to other abundantly available ligands such as amino and carboxylate groupings. Part of the problem in interpreting whole animal effects from our knowledge of molecular level effects lies in our difficulties of studying the properties of a small number of ligands which may be involved in metal binding.

An excellent example of the importance of a critical sub-group of ligands of the same type is the case of metal binding to membrane-bound ligands. The ability of the metal to interact with such ligands is determined not only by chemical specificity but also by what Rothstein has described as "geographical" specificity. Chemical specificity may be equated to the affinity constant of the metal for the ligand. Geographical specificity is the ability of the cation to diffuse to ligands buried within the membrane.

Several principles govern the interaction of metals with cellular ligands. The outer surface of the cell membrane will be the first part of the cell to come into contact with the metal. Heavy metals that penetrate the cell membrane very quickly, such as methylmercury cations, probably undergo very little reaction with membrane components. On the other hand, those metals which pass through the membranes slowly and are able to form tight bonds with ligands might seriously affect membrane function. In general, many more metal binding sites will be found in the interior of the cell than in the membrane. The possibility of protection in terms of metal binding to non-specific sites is less in the case of interaction with the membrane than in the case of interaction with components within the cell.

An important example of geographical specificity has been illustrated by Rothstein in the interaction of organomercurial cations with the sulfhydryl ligands in the red cell membrane (for review see Rothstein, 1973). The organomercurial chlormerodrin labelled with a radioisotope of mercury was allowed to react for a brief period of time with a suspension of red cells at a low temperature. The organomercurial proved to react only with sulfhydryls on the outer surface of the cell membrane. Quantitative aspects of this finding are indicated on the Scatchard plot given in Figure 5. A graph of the bound over the free organomercurial versus the bound mercurial gives a linear relationship over most of the concentration range. The Scatchard equation has found extensive use in studies of metal binding to protein and, more recently, of metal binding to cell membranes. The slope of the line is a measure of the affinity constant of the metal for the ligand and the horizontal intercept is the measure of the number of ligands per protein or per red blood cell. The number of SH groups bound to chlormerodrin in this example is less than 1% of the total SH groups inside the red cell and represents only a fraction of those SH groups in the entire membrane. Reaction of chlormerodrin with this small number of SH surface groups results in the inhibition of sugar transport across the red cell as shown in Figure 6.

Reaction with the surface groups has no effect on the potassium transport properties of the red cell membrane. Only in the case where an organomercurial penetrates within the structure of the membrane itself is potassium permeability affected.

The point of these studies is to illustrate that geographical specificity may be just as important as chemical specificity in determining the selective action of a metal. They also indicate that there may be time-dependent effects of metals as the metal cation diffuses from the outside to the interior of the cell.

The interaction of metals with membranes within the cell such as those in the mitochondria and microsomes is undoubtedly governed by similar principles as those described by Rothstein for the plasma membranes of cells. Aldridge (1970) was able to show that in mitochondria the cation triethyltin was probably bound to histidine residues. The interaction of triethyltin with mitochondrial membranes leads to uncoupling of oxidative phosphorylation.

Webb (1977) has discussed examples where divalent metal cations can displace calcium from the nerve membranes at neuromuscular junctions. Heavy metals may also react with SH groups on the nerve membrane, thus affecting the transport of calcium. The effects of many of the metals on the central nervous system could well be mediated through their affecting the activity of receptors to hormonal transmitters such as acetyl choline, as has been demonstrated in the case of methylmercury.

Acknowledgement
This paper is based on work performed under a Center grant (ES 01247) and Program Project grant (ES 01248) from the National Institute of Environmental Health Sciences and under contract with the U.S. Energy Research and Development Administration as a part of the University of Rochester Biomedical and Environmental Research Project. It has been assigned report no. UR-3490-1253.

REFERENCES

Aldridge, W.N. (1970). In: "Effects of Metals on Cells, Subcellular Elements and Macromolecules." (J. Maniloff, J.R. Coleman and M.W. Miller, eds) pp 255-271. Charles C. Thomas, Springfield, Ill.

Aub, J.C., Fairhall, L.T., Minot, A. and Reznikoff, P. (1926). "Lead Poisoning." Williams & Wilkins, Baltimore.

Clarkson, T.W. (1972). "Recent Advances in the Toxicology of Mercury with Emphasis on the Alkylmercurials." Vol. II, pp 203-234. CRT, CRC Press, Cleveland.

Clarkson, T.W. (1976). In: "Trace Elements and Human Disease." (A. Prasad, ed) pp 453. Academic Press, New York.

Clarkson, T.W. and Toole, S.R. (1964). Amer. J. Physiol. 206, 658-668.

Clarkson, T.W. and Vostal, J.J. (1973). In: "Modern Diuretic Therapy in the Treatment of Cardiovascular Disease." (G. Wilson and A.F. Lant, eds) pp 221-232. Excerpta Medica Foundation, Amsterdam.

Clarkson, T.W., Gatzy, J. and Dalton, C. (1961). Studies on the equilibration of mercury vapor with blood. University of Rochester Atomic Energy Project, Rochester, N.Y., Report No. UR-582.

Gage, J.C. (1964). Brit. J. Ind. Med. 21, 197-202.

Magos, L. and Butler, W.H. (1976). Arch. Toxicol. 35, 25-39.

Magos, L., Halbach, S. and Clarkson, T.W. (1977). The role of catalase in the oxidation of mercury vapor. Biochem. Pharmacol. (In press).

Miller, T.B. and Farah, A.E. (1962). J. Pharmacol. Exp. Therap. 135, 102-111.

Nielsen-Kudsk, F. (1965). Acta Pharmacol. Toxicol. 23, 263-274.

Norseth, T. (1971). Acta Pharmacol. Toxicol. 30, 172-176.

Norseth, T. (1973). In: "Mercury, Mercurials and Mercaptans." (M.W. Miller and T.W. Clarkson, eds) pp 264-272. Charles C. Thomas, Springfield, Ill.

Norseth, T. and Brendeford, M. (1971). Acta Pharmacol. Toxicol. 29, 375-384.

Norseth, T. and Clarkson, T.W. (1971). Arch. Environ. Health 22, 568-577.

Passow, H., Rothstein, A. and Clarkson, T.W. (1961). Pharmacol. Rev. 13, 185-224.

Riggs, A.F. (1952). J. Gen. Physiol. 36, 1-16.

Rossman, T.M., Meyn, S. and Troll, W. (1977). Effects of arsenite and DNA repair in Escheria Coli. Environ. Health Perspect. (In press).

Rothstein, A. (1973). In: "Mercury, Mercurials, and Mercaptans." (M.W. Miller and T.W. Clarkson, eds) pp 68-92. Charles C. Thomas, Springfield, Ill.

Rustam, H., Von Burg, R., Amin-Zaki, L. and Elhassani, S. (1975). Arch. Environ. Health 30, 190-195.

Shamoo, A.E., MacLennan, D.A. and Eldefrawi, M.E. (1976). Chem. Biol. Int. 12, 41-52.

Stock, A. (1934). Z. Anorg, Chem. 217, 241-253.

Surtshin, A. (1957). Amer. J. Physiol. 190, 271-277.

Surtshin, A. and Yagi, K. (1958). Amer. J. Physiol. 192, 405-409.

Turner, M.D., Smith, J.C., Kilpper, R.W., Forbes, G.B. and Clarkson, T.W. (1975). Paper presented at the International Conference on Heavy Metals in the Environment. Toronto, Canada, Oct. 27-31.

Vander, A.J. (1962). Amer. J. Physiol. 203, 1005-1007.

Vostal, J.J. and Clarkson, T.W. (1973). In: "Mercury, Mercurials, and Mercaptans." (M.W. Miller and T.W. Clarkson, eds) pp 139-155. Charles C. Thomas, Springfield, Ill.

Webb, M. (1977). In: "Clinical Chemistry and Chemical Toxicology of Metals." (S.S. Brown, ed) pp 51-64. Elsevier, Amsterdam.

Weiner, I.M., Levy, R.I. and Mudge, G.H. (1962). J. Pharmacol. Exp. Therap. 138, 96-112.

__Table 1__. Dissociation constants (negative logarithm) for complexes of methylmercuric and mercuric ions (From Clarkson, 1972).

Ligand	$CH_3 - Hg^+$	Hg^+
Cl	5.4	6.7
OH	9.5	10.3
Histidine (NH_2)	8.8	10
Cysteine	15.7	14
Albumin	22.0	13

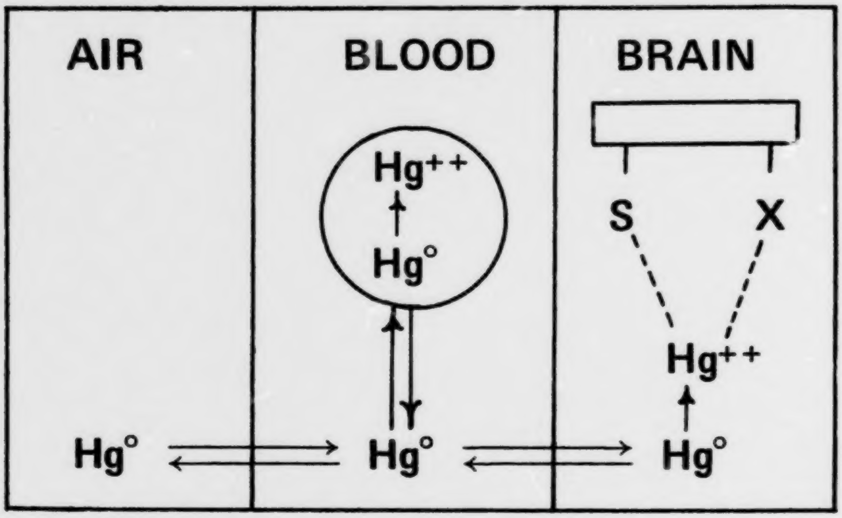


Figure 1. A schematic representation of the metabolism of inhaled mercury vapor. The vapor rapidly crosses the pulmonary membranes and enters the plasma compartment. From this location it may enter the red blood cells where it undergoes oxidation to form divalent ionic mercury. The latter binds to cellular ligands. Elemental mercury may also cross the blood-brain barrier where it is oxidized, resulting in tissue binding of ionic mercury. Mercury vapor dissolved in plasma is believed to persist for sufficient time to allow diffusion into all tissues in the body including passage across the placenta and uptake by the fetus (From Clarkson, 1976).

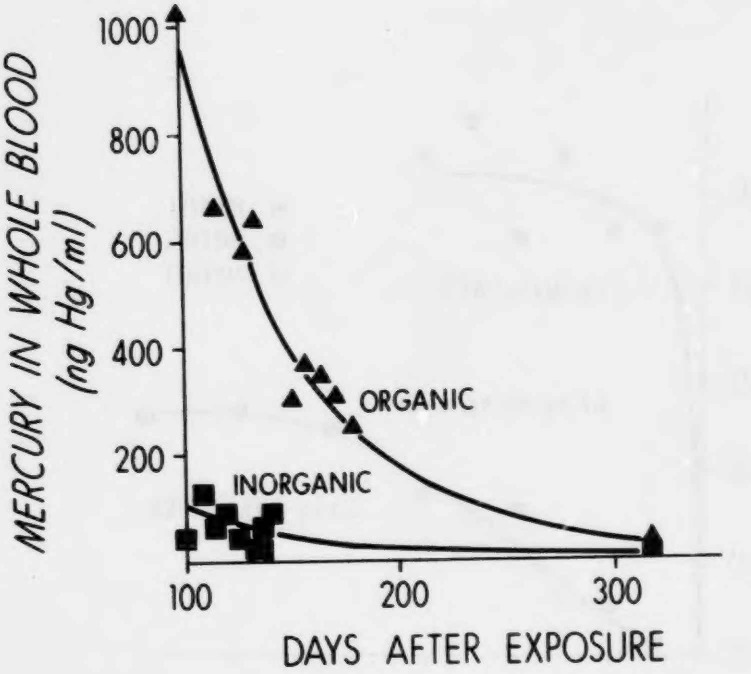


Figure 2. The concentration in whole blood of organic and inorganic mercury in a person after dietary exposure to methylmercury.

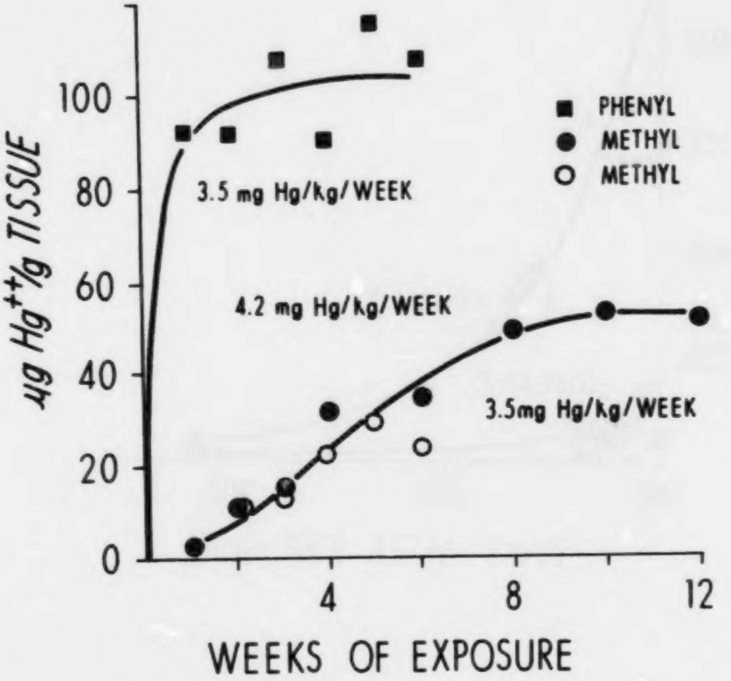


Figure 3. The accumulation of inorganic mercury in rat kidneys following repeated doses of phenyl or methylmercury compounds (Data from Gage, 1964, and Magos and Butler, 1976).

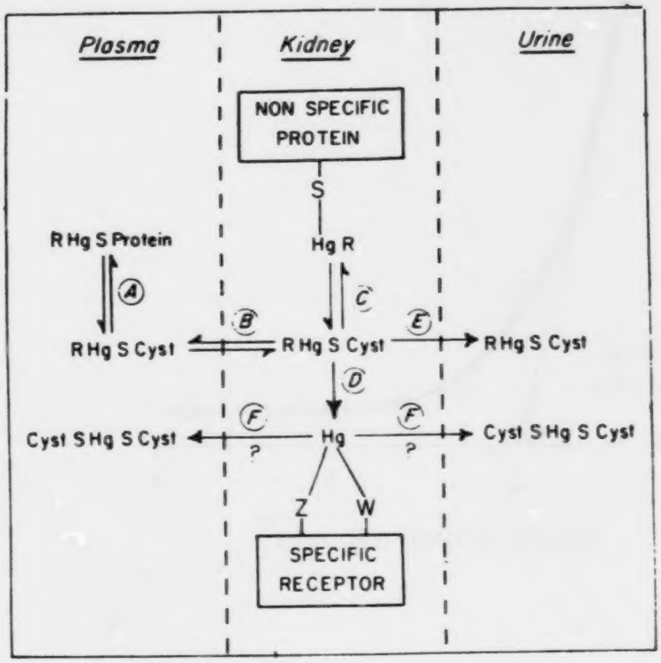

Plasma
Kidney
Urine
NON SPECIFIC PROTEIN
S
Hg R
R Hg S Protein
A
B
C
E
D
F
?
R Hg S Cyst
R Hg S Cyst
R Hg S Cyst
Cyst S Hg S Cyst
Hg
Cyst S Hg S Cyst
Z
W
SPECIFIC RECEPTOR

Figure 4. A diagram of the action of mercurial diuretics on kidney tissue. For further description, see text (From Weiner et al., 1962).

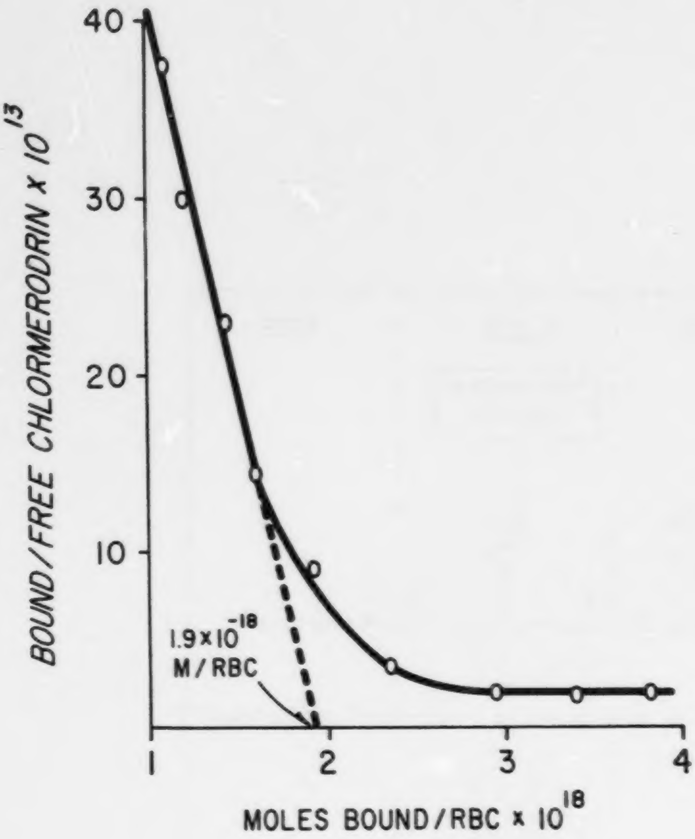


Figure 5. A mass law (Schatchard) plot of the binding of chlormerodrin by human red blood cells (From Rothstein, 1973).

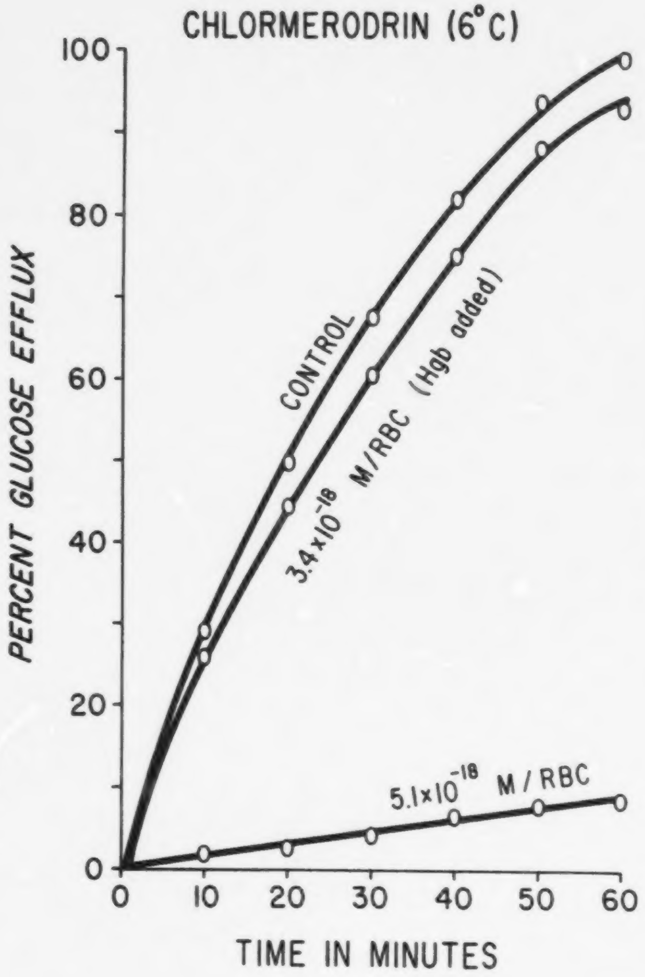


Figure 6. The reversal of the inhibition of chlormerodrin on glucose efflux from human red blood cells as related to the binding and desorption of the agent (From Rothstein, 1973).

FACTORS INFLUENCING EFFECTS AND DOSE-RESPONSE RELATIONSHIPS OF METALS

Gunnar Nordberg, Jiri Parizek and Magnus Piscator

There is growing evidence that absorption, metabolism and/or effects of both essential and non-essential metals can be substantially influenced by various factors. The presence or absence of such factors may sometimes modify the character of effects and in other instances change dose-effect and dose-response relationships. These modifying factors include certain characteristics of the exposed organism, e.g. constitutional factors like sex and age, which often are called host factors. Absorption, metabolism, and effects of metals can also be modified by simultaneous or previous exposure to certain environmental agents. In some cases such agents exert their effect through a direct interaction with the metal in question at the molecular level. In other cases an indirect metabolic change is induced by the interfering environmental factor which gives rise to the change in dose-response relationship. Such indirect interactions are sometimes of considerable interest. However, it should be recognized that in certain cases, even where the influence of a certain environmental factor is well established, the corresponding mechanism may not be known. The term "interaction" may in such instances be used in a broad sense. Factors effectuating modifications of metal toxicity may range from direct interactions between a metal and another metal, e.g. cadmium-zinc interaction, to the influence of socio-economic and behavioral factors on toxic effects of metals.

For several decades chemical and physical interactions of metals have been studied in experiments both in vitro and in vivo in animals. There is an abundance of data on such interactions and no attempt will be made in this chapter to review all these data. Many results from such studies have found practical application in the field of animal nutrition, but their significance for imbalance of trace elements and for toxic effects of

Preceding page blank

some metals in the human being has been difficult to evaluate. Only such data that are of general interest and data that are thought to have a bearing on the human situation will be taken up. The experimental work has usually been performed on healthy animals of the same strain in carefully controlled environments. Often the doses or exposure routes have been of little relevance to the human situation. Nevertheless such studies may sometimes give indications about what might happen in human beings and may be a basis for further studies with more relevance to human beings.

At a meeting in Tokyo, 1974 (Nordberg, 1976) some results from such animal studies were discussed and several interactions of possible importance for human beings were recognized. Further considerations concerning interactions among metals and other factors of importance in metal toxicology were given during a meeting held in Stockholm, 1977, by the Task Group on Metal Interactions (Nordberg, 1978). At the Tokyo meeting interaction was defined as a process by which metals in their various forms, or other factors, change the critical concentration or a critical effect of a metal under consideration. At the Stockholm meeting this definition was accepted with the addition that the term interaction should also be used to describe the influence of other substances and factors on the metabolism and toxicity of metals (Task Group on Metal Interaction, 1978).

1. Definitions

As mentioned above the factors that will be discussed in this chapter and which can influence metal metabolism and toxicity include both host factors and external factors like climate etc., but the main part of the document will be concerned with interactions between metals and other metals or chemicals. Such interactions may be both direct and indirect, in which case a distinction may be made between "non-interactive" and "interactive" joint action.

"Independent action" is one type of non-interactive joint action. This is by far the most common type and occurs when two metals have different sites of action or different modes of action, and when they do not influence each other. Another example of non-interactive joint action is when two metals exert action at the same site and their modes of action are similar but they do not influence the action of each other. The result of such joint action is an addition of effects which can be estimated from the dose-effect and dose-response relationships of the constituents of a mixture. Such a type of joint action is the one of arsenic and lead in relation to coproporphyrin excretion (Fowler, 1978).

In addition to these non-interactive types of joint action of chemicals there are other types of interactions where the dose-effect or dose-response curve of the combination cannot be assessed from those of the individual chemicals.

Synergism takes place when the effect or response of the combined exposure is greater than additive. Examples are the synergistic effects of cigarette smoking and asbestos in inducing lung cancer. An example involving metals is the greatly increased teratogenic effect seen in animals from the combined injection of cadmium and lead in relation to the effects of each of the metals separately.

Antagonism takes place when one factor reduces the effect of another. An example of relevance for this chapter is the interaction between selenium and various forms of mercury which will be discussed in more detail later (section 2.4).

With regard to the joint action of metals or metals and other chemicals, the type of interaction will be classified as additive, synergistic or antagonistic, which are common concepts in pharmacology, whenever possible in the text to follow.

Some of the mentioned types of combined action do not apply exclusively to chemicals but also to other exposure factors, such as ionizing radiation, climatological factors, and infectious agents. A synergistic action of smoking and exposure to radon has been known to increase the risk for lung cancer. In animal experiments, variations in temperature have been shown to cause great differences in the LD_{50} of one and the same chemical.

When it comes to such host factors as age, sex, race and other genetic factors it is not appropriate to talk about an interaction. Nevertheless, it is well recognized that such factors influence dose-effect and dose-response curves of metals. Such factors will therefore be included in this chapter.

Since data on interactions of other factors in principle can be found in most papers dealing with metal toxicity, it is obvious that it would be an enormous task to extract all the relevant data. Only in a few instances can it be seen directly that a report is concerned with interaction. This chapter will therefore focus on metal-metal interactions and take up several of the above mentioned factors rather briefly, even though they are probably of very great importance.

The specific information given in this chapter relies to a large extent on data and conclusions presented at the meeting in Stockholm and reported by the Task Group on Metal Interaction (Nordberg, 1978). That group also discussed some special cases of interactions like those resulting from suboptimal intakes of certain nutrients, but this will not be regarded as a separate category here.

2. Metal-metal interactions

These interactions have long been a subject of study in the field of animal nutrition. It was recognized early that livestock had to have a carefully controlled intake of essential elements in order to ensure high food standards for humans (see e.g.

Underwood, 1977). During later years the need has arisen to extend the observations in this field to non-essential elements since the increase in pollution in many areas of the world has caused an unwanted exposure to toxic metals. Studies on effects of metals on human beings, both in the general and the industrial environment, have given us an awareness that simultaneous exposure to several toxic elements might occur and that the intake of essential elements might at the same time be marked by deficiencies. Presently of special concern with regard to the human situation are arsenic, cadmium, lead and mercury, which were discussed in some detail at the Stockholm meeting (Nordberg, 1978). In the following the present experience with regard to these metals and some other metals of interest will be summarized. Data on specific interactions will also be found in the chapters on the specific metals in Volume II of this series.

2.1 Interactions between arsenic and other metals

Special attention has been devoted to selenium since both arsenic and selenium can form similar types of ions, for which reason antagonism could be expected. Both metals can be methylated *in vivo* (Braman and Foreback, 1973; Francis et al., 1974) which may complicate the understanding of possible interactions. It has been shown that arsenic can decrease the toxicity of selenium, one mechanism being that arsenic increases the excretion of selenium via bile (Levander and Baumann, 1966a, 1966b). On the other hand, injection of arsenic compounds was found to increase the toxicity of methylated selenium compounds (Obermeyer et al., 1971). It is also known that selenium in turn will increase the biliary excretion of arsenic (Levander and Baumann, 1966a) and that high dietary levels of selenium cause decreases in organ concentrations of arsenic (Whanger, 1976).

There are, however, no human data which can support these findings in animals. It is thus not known at present to what extent these interactions are of importance in human beings. Simultaneous exposure to selenium and arsenic may occur in industrial environments. Certain groups of the general population are exposed to large amounts of arsenic from drinking water or to methylated arsenic compounds in marine organisms. The selenium intake of such groups could be of importance for dose-response relationships for arsenic.

The influence of cadmium and lead on the toxicity of arsenic has been investigated in a few animal experiments. It was found that lead additively increased protoporphyrin excretion in arsenic-treated animals, whereas cadmium had no effect (Mahaffey and Fowler, 1977). Since the doses were high it is impossible to draw any conclusions from these studies with regard to the human situation. A combination of cadmium, lead, and arsenic is not uncommon in certain industrial environments.

2.2 Interactions between cadmium and other metals

That zinc can counteract many of the toxic effects of cadmium has been shown in a large number of studies. Cadmium and zinc belong to the same group in the periodic table. It has been speculated that cadmium can displace or replace zinc in some essential systems in the organism, thus causing functional changes. Especially some parts of the reproductive system have very high concentrations of zinc and many of the sexual functions require zinc. Large amounts of cadmium in single doses may cause testicular destruction, but this damage may be prevented by injection of large amounts of zinc (Parizek, 1957). In many of the earlier studies on relationships between zinc and cadmium (see e.g. review by NAS, in press), animals exposed to cadmium were on diets with extremely high concentrations of zinc, which might have prevented some of the expected effects of cadmium from appearing. Of greater interest are the studies where animals have been exposed to cadmium, but where the dietary concentrations of zinc have been just adequate or suboptimal. In such experiments it has been shown that exposure to small amounts of cadmium may cause a redistribution of zinc in the organism (Petering et al., 1971). Zinc concentrations increase in liver and kidney and might decrease in other organs where cadmium does not accumulate, e.g. testes. When the intake of zinc was increased, these decreases in organs were not seen. It has also been shown that if pregnant animals are exposed to cadmium, the fetal concentrations of zinc as well as of copper may be lowered (Pond and Walker, 1975; Choudhury et al., 1977). It could be expected that cadmium would cause changes in the activities of some enzymes which require zinc. It has been shown that the renal activity of leucine aminopeptidase, which is a zinc-requiring enzyme, will be reduced in animals exposed to cadmium (Cousins et al., 1973). It has, however, not been shown that cadmium actually replaces zinc in any enzyme *in vivo*.

That the increase in cadmium concentrations in the kidney with age is accompanied by an equimolar increase in zinc concentrations has been shown in studies on human beings (Piscator and Lind, 1972; Elinder et al., 1977). In other words, for each atom of cadmium that enters the kidney, one atom of zinc will also be stored. There are indications that above a certain level, corresponding to about 60 mg Cd/kg wet weight in renal cortex, this equimolar increase of zinc does not go on. A change in the cadmium-zinc relationships at higher levels of cadmium is thus suggested. This might be a factor of importance for development of renal damage in cadmium-exposed people. This change in the equimolar relationship has also been found in studies on horses (Piscator, 1974; Elinder and Piscator, 1977). Since the intake of zinc in human beings is generally regarded as being only optimal, or in certain population groups suboptimal, it is obvious that excessive exposure to cadmium may cause changes in zinc metabolism and perhaps also a lowering of zinc concentrations in some organ systems. There might also be a risk for lower zinc concentrations in the fetus if pregnant women are exposed to cadmium.

With regard to copper, several animal experiments, mainly on the rat, have given data (Petering, 1974; Stonard and Webb, 1976; Bremner and Campbell, 1978) which show that cadmium can cause changes in its distribution. Thus, exposure to cadmium caused increases in the renal concentrations of copper, which could be attributed entirely to an increased content of copper in the cadmium binding protein, metallothionein (Stonard and Webb, 1976). It has been shown in animals that the copper concentrations in the fetus may be reduced if the mother is exposed to cadmium. At present there are no data that show that cadmium has a profound influence on copper metabolism in human beings (Bremner and Campbell, 1978). In contrast to the zinc levels in kidney, which are related to the cadmium levels, the copper levels in the human kidney are fairly constant during a life-time and do not seem to be related to the cadmium exposure (Schroeder et al., 1966; Piscator and Lind, 1972; Tsuchiya and Iwao, 1978).

Cadmium can cause anemia, one mechanism for this action being a decreased absoprtion of iron from the gut. This anemia can be reversed by injections of iron compounds (Berlin and Friberg, 1960). It has also been shown that iron deficiency may increase the absorption of cadmium from the gut (Hamilton and Valberg, 1974; Valberg et al., 1976). There are, however, no indications of direct interactions between cadmium and iron in the body. An indirect interaction is the change in iron metabolism caused by hemolysis in cadmium-exposed rabbits (Axelsson and Piscator, 1966).

Interactions between cadmium and calcium have attracted great interest during later years, one reason being the osteomalacia occurring in cadmium exposed women in Japan, the so-called Itai-itai disease (see Friberg et al., 1974). In animals on calcium deficient diets, the absorption of cadmium will increase, and vice versa, i.e. in animals with high dietary levels of calcium, the absorption of cadmium as well as of several other metals will decrease. It has been proposed that cadmium may be bound to the calcium-binding protein which is responsible for the uptake of calcium from the mucosal wall. With regard to systemic action, it has not been possible to show any direct effect of cadmium on bone or on calcium metabolism. It has, however, been suggested that at high renal concentrations of cadmium, cadmium might interfere with the synthesis of vitamin D_3 in kidney, thus indirectly influencing bone metabolism.

With regard to interactions between cadmium and some other toxic metals of interest, especially arsenic, lead, and mercury, very few attempts have been made to study these problems. It is known that mercury can bind to the cadmium-binding protein metallothionein, and that interactions may thus occur in the kidney between these two metals (Magos et al., 1974). Mercury has a stronger binding capacity and could displace cadmium from metallothionein. Lead does not bind to metallothionein *in vivo*. There are no data showing any direct interactions between lead and cadmium, or between arsenic and cadmium. No data have yet been put forth which would indicate any important relationships between these metals in human beings even though these metals often occur simultaneously in the environment.

In summary, the present data suggest that the most important interactions in which cadmium is involved in human beings concern zinc. Animal data suggest that important interactions may occur between cadmium and copper in certain animals. It is recommended that whenever cadmium is analyzed for evaluating human exposure conditions, primarily zinc but also copper should be analyzed.

2.3 Interactions between lead and other metals

A decrease in the activity of the enzyme ALA-D, which is a zinc-requiring enzyme, in the presence of a high exposure to lead has been shown in both humans and animals. Simultaneous administration of zinc can prevent this decrease in activity. Horses exposed to lead through industrial pollution showed less severe responses when there was also excessive exposure to zinc (Willoughby et al., 1972). Important interactions, the most so in the gut, occur between lead and calcium, the similarity between these metals making bone the main storage organ for lead. Calcium deficiency has been demonstrated to increase the absorption of lead in several experiments. It has also been indicated in human studies that especially people with high absorption of calcium, e.g. children, have a higher absorption of lead (Alexander et al., 1973). It may be noted that some of the neuro-muscular actions of lead might be due to a direct interference with binding sites usually occupied by calcium (Silbergeld et al., 1974).

Iron deficiency has been shown to cause an increase in the absorption of lead. Lead can cause anemia, but this is due to the interference of lead with the synthesis of hemoglobin and to some extent to intravascular destruction of red cells. No direct interactions between lead and iron in the body have been demonstrated.

There are no data on any interactions between lead and mercury. With regard to interactions between lead and arsenic, reference is made to the section on arsenic (section 2.1) where some animal data are presented. In summary, the absorption of lead ingested with food might be influenced to a great extent by the calcium and iron status. There are also data indicating important interactions between zinc and lead.

2.4 Interactions between mercury and its compounds and other metals

It has been recognized that data are limited with regard to the influence of metals and metalloids on the toxicity of inorganic mercury and that definite conclusions with regard to humans cannot be given (Task Group on Metal Interaction, 1978). Some interesting animal data (review: Parizek, 1972; Ganther et al., 1973; Parizek et al., 1974; Levander, 1978) will be mentioned in the following since they may have possible relevance for humans as well.

Selenite and other compounds that are metabolized to selenite have been shown to reduce the lethal effect of inorganic mercury in rats, preventing the development of renal tubular and intestinal necrosis (Parizek and Ostadalova, 1967; Parizek et al., 1971). Dietary selenate prevents the depression of growth in rats receiving mercuric chloride by mouth (Potter and Matrone, 1974). In long-term experiments dietary selenate reduces the chronic renal tubular damage produced by oral mercuric chloride in rats (Groth et al., 1973, 1976).

Parizek et al. (1969, 1974) demonstrated that administration of selenite gave rise to a marked increase in mercury in blood and a reduced excretion of mercury. Both mercury and selenium were bound to a plasma protein with an atomic ratio of one (Burk et al., 1974). The formation of a mercury-selenium-protein complex may explain these alterations in the kinetics and toxicity of mercury (Parizek et al., 1971, 1974, Parizek, 1976) and may also be related to the observation by Groth et al. (1976) of intranuclear inclusion bodies containing Hg and Se. The decreased passage of mercury and selenium across the placenta and into milk when inorganic mercury and selenite are administered simultaneously may also be explained by the formation of an Hg-protein-Se-complex.

An indication that similar processes as those demonstrated in animals might occur in man is provided by the observations by Kosta et al. (1975) of elevated concentrations of mercury and selenium in organs of mercury miners many years after cessation of exposure.

The influence of other metals on inorganic mercury toxicity has not been documented to any noteworthy degree in the literature. One observation is that pretreatment of animals with cadmium protected (male) rats from the nephrotoxic effect of inorganic mercury (Magos et al., 1974). This action is regarded to be mediated through an induction of metallothionein.

With regard to organic mercury compounds, the Task Group on Metal Interaction (1978) considered that the toxic effects of methylmercury had been shown to be influenced by selenite in several animal species. Thus 0.5-8.0 mg/kg of selenite in the diet protected chicks, quail and rats from the toxic effects of up to 0.2 mmoles/kg of methylmercury in the diet. Although it has been suggested that selenium present in marine fish might protect against the toxicity of dietary methylmercury (Ganther et al., 1972; Ganther and Sunde 1974; Ohi et al., 1976) the evidence for this is inconclusive; for instance the difference in toxicity could be explained by differences in methylmercury intake, or in quantity and quality of protein in the diet (Stillings et al., 1974).

Dietary selenite and vitamin E diminished the mortality in methylmercury exposed quail (Welsh and Soares, 1976). In rats Welsh (1976) showed that vitamin E and other antioxidants had a protective effect.

In a study of humans exposed to methylmercury, multiple regression statistical analysis revealed an inhibition of ALA-D in red cells related to concentrations of both mercury and lead in blood (Schütz and Skerfving, 1975). The molar effects of mercury and lead were similar.

On basis of the evidence available, the Task Group on Metal Interaction (1978) found that selenite alters the kinetics and reduces the neurotoxicity of methylmercury in several animal species, at least at high levels of methylmercury exposure. Methylmercury also alters selenium kinetics in rats.

2.5 Interactions between copper and other metals

The influence of cadmium on copper metabolism has already been mentioned. Another important relationship is that between molybdenum and copper. This is of enormous importance for ruminants where very small changes in the concentration of any of these metals may cause disease. An excessive intake of molybdenum may cause copper deficiency. Data on human beings are scarce but Kovalskii et al. (1961) have reported that a high dietary intake of molybdenum caused increases in the urinary excretion of copper and a lowering of blood copper levels. In an experimental study on human beings it was shown that an increase in the dietary intake of molybdenum caused an increase in the excretion of copper (Deosthale and Gopalan, 1974). Animal data indicate that important relationships might exist between copper and zinc. It has been proposed that the zinc/copper ratio in the diet could be related to cardio-vascular diseases in human beings (Klevay, 1975). Oral therapy with zinc sulfate in large doses (150 mg Zn/day) has recently been shown to cause copper deficiency in a patient treated for coeliac disease (Porter et al., 1977).

2.6 Interactions between thallium and potassium

Thallium and potassium seem to have some common receptor sites where they compete and thus undergo direct interaction. Potassium has been proposed for use in the treatment of thallium poisoning since the administration of potassium increases excretion of thallium. For further data see Kazantzis (1977).

3. Interactions between metals and nutritional factors

In addition to the nutritional factors which have already been mentioned, e.g. the intake of some metals, other nutrition components might have a profound influence on metal metabolism. It is well-known that phytate in certain diets causes a reduction in the absorption of zinc and renders zinc less available from vegetable diets than from diets based mainly on meat. The protein content of the diet has been shown to play a role in cadmium and lead toxicity, i.e. the less protein, the higher absorption and the more severe the signs of toxicity. Of special interest is that milk will increase the absorption of several metals considerably. In neonatal and adult animals Kostial and co-workers (e.g. Kello and Kostial, 1973; Kostial et al., 1975), have found that a milk diet causes a very high absorption of certain elements like lead and cadmium and that the proportion of milk in the diet can be directly related to the absorption of certain metals. The factor mainly responsible for this high absorption has not yet been identified but the lipid content has been put forth in several contexts as one such factor.

Among the vitamins, vitamin C has been shown to reduce lead (Clegg and Rylands, 1966) and cadmium toxicity (Fox, 1975), but on the other hand vitamin C increases the retention of mercury in the tissues (Blackstone et al., 1974). Human data are scarce, but it has been indicated that one reason for the Itai-itai disease was low intakes of calcium, vitamin D, and protein (Friberg et al., 1974).

4. Influence of drugs on metal metabolism and toxicity

Among the drugs taken by healthy people at large are oral contraceptives. Intake of such synthetic hormones is known to cause changes in copper metabolism, influencing the synthesis of ceruloplasmin and bringing about higher serum concentrations of copper. Zinc metabolism is also recognized to be influenced by oral contraceptives. It has not been established as to whether exposure to toxic metals like lead and cadmium might need to be evaluated by different dose-response curves in women taking such pills. Some of the drugs for treatment of hypertension, which essentially have a chelating action, also affect metal metabolism. Prolonged treatment with some of these drugs was noted to increase zinc excretion, but not cadmium excretion (Wester, 1974). Such treatment may thus in the long run cause changes in the cadmium-zinc relationship which may be of importance especially in people with a high body burden of cadmium. To what extent antibiotics might change metabolism of both essential and non-essential metals has not been documented in detail, but one animal study has indicated that penicilline might cause changes in the distribution of cadmium (Dequidt et al., 1973).

5. Influence of other chemicals on metal toxicity

Ethyl alcohol has been shown to cause an increase in the absorption of lead. This might be of weight when evaluating dose-effect relationships in people exposed to lead in illicitly distilled alcohol. In cases of lead poisoning caused by such consumption, the symptoms from the CNS seem to be more severe than what could be expected from lead exposure alone. Some synergistic effects between alcohol and lead might thus be suspected.

Consumption of large amounts of alcohol can temporarily depress ALAD activity, which is also a sign of lead toxicity. However, it has also been shown that ALAD activity may increase in animals exposed to both lead and alcohol. The depression caused by either of the substances alone is partially reversed when the two are combined (Moore, 1975).

Alcohol influences the metabolism of mercury after exposure to mercury vapor. This is thought to be due to the interference of the alcohol with some of the enzymes taking part in the conversion of mercury vapor to bivalent mercury (Nielsen-Kudsk, 1965). Simultaneous exposure to cobalt and alcohol has caused the so-called beerdrinker's cardiomyopathy, where the effect was greater than what could be expected from the single action of the compounds (see Elinder and Friberg, 1977).

Exposure to 50 ppm carbon monoxide, a level not unusual in industrial operations, caused changes in the distribution of some essential metals in animal experiments (Mazaleski et al., 1970). It is not known to what extent this might apply to human beings and to exposure to toxic metals.

6. Influence of age on metal toxicity

It has long been claimed that certain age groups are subject to special risks, i.e. young children and elderly. However, not until recently have quantitative data been available to document these claims. Several investigations have dealt with young animals and children, whereas data on elderly people are still lacking. The fetus is regarded as specially vulnerable since many functions are not fully developed until after birth, and several possibilities exist for contact between the fetus and toxic metals. Especially methylmercury is known to cross the placental barrier and accumulate in the fetus. That fetal exposure to methylmercury can cause intrauterine methylmercury poisoning is well documented. Lead crosses the placental barrier, but it is still a subject of discussion as to what extent pre-natal lead exposure plays a role in later development. It is suspected that the fetal brain does not tolerate lead to the same extent as does the adult brain. The data available at present do not permit any certain conclusions.

Extensive studies by Kostial and co-workers have shown that the neonate animal has a higher absorption of certain toxic metals like lead and cadmium due to its dependence on the milk diet (see section 3). The neonate also has a higher whole body retention, higher blood levels, and a much higher accumulation in the brain compared to the adult exposed to similar doses. Exposure to lead in the early neonatal stages in animals can cause changes in emotional behavior, learning deficits and increased motor-actovity. There are also data suggesting that children develop symptoms at lower blood lead concentrations and have a higher absorption of lead than do adults. The behavioral effects noted in animals have also been observed in children. With regard to the age group 10-20 years, there are not data available. Since the greatest calorie intake is required during that period, it follows that the highest amounts of e.g. cadmium will be accumulated at the same time.

7. Influence of sex on metal toxicity

When women are discussed as a risk group what is often actually meant is that through them the fetus may be exposed. The sex differences to be discussed here concern only those directly applicable to sex itself.

Data from animal experiments are to some extent conflicting. Lead acetate (Kostial et al., 1974) and cadmium chloride (Engström and Nordberg, 1978) have been shown to have a higher acute toxicity in male than in female animals. On the other hand, female rats were shown to absorb twice as much cadmium after a single oral dose as male rats (Kello et al., 1978). Since castrated males absorbed as much as the females, it was then concluded that male sex hormones were responsible for the decreased retention of cadmium in males.

8. Influence of physical factors on metal metabolism and toxicity

Since sweat is an important excretion route for some metals, e.g. zinc and nickel, it can be expected that in hot climates or in hot industrial environments metal losses via sweat might influence metabolism and toxicity. Workers exposed to nickel in a hot environment had considerably lower nickel levels in blood than expected (Szadkowski et al., 1969). Zinc losses via sweat would be of special importance in persons also exposed to cadmium.

High noise levels are common in many industrial environments and prolonged exposure to noise might indirectly cause some physiological changes which in turn might have an impact on metal metabolism.

9. Influence of irritant gases and lung function on metal metabolism and toxicity

The clearance of inhaled particles from the lung is dependent on the intact function of the mucociliary transport system. Long-term cigarette smoking and bacterial and viral diseases can impair this transport system. A decreased clearance of inhaled particles might increase the absorption of certain metals or prolong their retention time in the lungs. It is not known to what extent simultaneous exposure to irritant gases, like SO_2, and metals will affect the distribution and toxicity of these metals. Another part of the pulmonary defence system consists of the alveolar macrophage, the function of which might be depressed by exposure to nitrogen dioxide. This might result in prolonged retention time and thus a greater absorption of slowly soluble metallo-compounds in the alveoli. The metals themselves might have an effect on the macrophage function and thereby disturb the mucociliary transport system.

Smoking may affect the uptake and metabolism of metals in several ways. Long-term smoking might cause an impairment of the mucociliary transport system, as said above. Carbon monoxide might affect metal distribution, as also mentioned earlier. Since the smoker will inhale large amounts of carbon monoxide this could be another factor of importance. However, there are at present no data available on this matter in human beings.

Finally it should be mentioned that the cigarette smoke in itself contains certain metals of toxicological interest, e.g. cadmium, nickel, mercury, lead, and thus smoking itself might contribute to the metal burdens (see Volume II of this series under the specific metals for details).

10. Influence of personal hygiene on metal metabolism and toxicity

Both the industrial and the general environments present great possibilities for contamination of hands and clothing with metal compounds. In the industrial environment large amounts of metallic dust will accumulate and may be transferred to the human body through smoking, i.e. if cigarettes or pipe tobacco are contaminated, or to the mouth by direct contact with contaminated hands. Such circumstances might cause a considerable extra exposure, making it extremely difficult to arrive at dose-response relationships based on measurements of the actual metal in air. In a study at a Swedish cadmium factory, it was recently found that the cadmium levels in air measured by personal samplers varied on an average between 2 μg and 10 $\mu g/m^3$ in a group of 15 workers, while the fecal elimination of cadmium varied on an average between 30 and 2,800 μg/day, indicating a considerable exposure via the gastrointestinal tract in some cases (Adamsson et al., to be published). The highest values were found among smokers, and among people who were generally classified as having bad personal hygiene habits in their work. Children playing in areas with lead-containing dust or soil may easily contaminate their hands and spread their exposure considerably by licking them. Exposure to mercury vapor in many laboratories, dentists' offices etc. can be avoided by good hygiene and by care in handling mercury drops.

11. Conclusions

A multitude of factors must be taken into account when evaluations of toxic hazards of metals are to be made. Every human being has a special set of external and internal factors which might influence the distribution and effects of metals in the body. As far as essential metals are concerned, homeostatic mechanisms in absorption and excretion maintain them at their physiological levels regardless of relatively large variations in exposure. External factors which are not part of man's normal environment may cause profound changes, however, a widespread example being the changes in metabolism of certain essential metals caused by oral contraceptives. The exposure to toxic metals, today often to several simultaneously, in man is a result of great changes in the external environment due to industrial processes and to the general pollution of the environment. The extent to which these metals can interact with each other and, in the long run, cause changes in each other's metabolism and toxicity is not known. To which extent the combination of such metals with other environmental pollutants, i.e. certain gases, can give other dose-effect relationships than the expected is also a largely unanswered question.

REFERENCES

Alexander, F.W., Delves, H.T. and Clayton, B.E. (1973). In: "Environmental Health Aspects of Lead." pp 319-331. Published by the Commission of European Communities Directorate General for Dissemination of Knowledge, Center for Information and Documentation CID, Luxembourg.

Axelsson, B. and Piscator, M. (1966). Arch. Environ. Health 12, 374.

Berlin, M. and Friberg, L. (1960). Arch. Environ. Health 1, 478.

Blackstone, S., Hurley, R.J. and Hughes, R.E. (1974). Food Cosmet. Toxicol. 12, 511-516.

Braman, R.S. and Foreback, C.C. (1973). Science 182, 2147.

Bremner, I. and Campbell, J.K. (1978). In: "Factors Influencing Metabolism and Toxicity of Metals." (G.F. Nordberg, ed) Op. cit.

Choudhury, H., et al. (1977). Dietary cadmium: Embryotoxicity and neonatal behavioral effects. Annual Report of the Center for the Study of the Human Environment. University of Cincinnati, Cincinnati.

Cousins, R.J., Barber, A.K. and Trout, J.R. (1973). J. Nutr. 103, 964-972.

Deostahle, Y.G. and Gopalan, C. (1974). Brit. J. Nutr. 31, 351-355.

Dequidt, J., Haguenoer, J.M. and Fromont, B. (1973). Arch. Mal. Prof. Med. Trav. 34, 427-436.

Elinder, C-G. and Friberg, L. (1977). In: "Toxicology of Metals." Volume II, pp 188-205. Environmental Health Research Series, 600/1-77-022, Environmental Protection Agency, Research Triangle Park.

Elinder, C.G. and Piscator, M. (1978). In: "Factors Influencing Metabolism and Toxicity of Metals." (G.F. Nordberg, ed) Op. cit.

Elinder, C.G., Piscator, M. and Linnman, L. (1977). Environ. Res. 13, 432.

Engström, B. and Nordberg, G.F. (1978). Effects of detergent formula chelating agents on the metabolism and toxicity of cadmium in mice. Toxicology (In press).

Fowler, B. (1978). In: "Factors Influencing Metabolism and Toxicity of Metals." (G.F. Nordberg, ed) Op. cit.

Fox, M.R.S. (1975). Ann. N.Y. Acad. Sci. 258, 144.

Francis, A.J., Duxbury, J.M. and Alexander, M. (1974). Appl. Microbiol. 28, 248.

Friberg, L., Piscator, M., Nordberg, G. and Kjellström, T. (1974). "Cadmium in the Environment." CRC Press, Cleveland.

Ganther, H.E. and Sunde, M.L. (1974). J. Food Sci. 39, 1.

Ganther, H.E., Goudie, C., Sunde, M.L., Kopecky, M.J., Wagner, P., Sand-Hwan Oh and Hoekstra, W.G. (1972). Science 175, 1122-1124.

Ganther, H.E., Wagner, P.A., Sunde, M.L. and Hoekstra, W.G. (1973). In: "Trace Substances in Environmental Health - VI." (D.D. Hemphill, ed) pp 247-252. University of Missouri, Columbia.

Groth, D.H., Vignati, L., Lowry, L., MacKay, G. and Stokinger, H.E. (1973). In: "Trace Substances in Environmental Health - VI." (D.D. Hemphill, ed) pp 187-189. University of Missouri Press, Columbia.

Groth, D.H., Stettler, L. and Mackay, G. (1976). In: "Effects and Dose-Response Relationships of Toxic Metals." (G.F. Nordberg, ed) pp 527-543. Elsevier, Amsterdam.

Hamilton, D.L. and Valberg, L.S. (1974). Amer. J. Physiol. 227, 1033.

Kazantzis, G. (1977). In: "Toxicology of Metals." Volume II, pp 388-404. Environmental Health Research Series, 600/1-77-022, Environmental Protection Agency, Research Triangle Park.

Kello, D. and Kostial, K. (1973). Environ. Res. 6, 355.

Kello, D., Dekanic, D. and Kostial, K. (1978). Influence of sex and dietary calcium on intestinal cadmium absorption in rats. Arch. Environ. Health (In press).

Klevay, L.M. (1975). Perspect Nutr. 28, 764.

Kosta, L., Byrne, A.R. and Zelenko, V. (1975). In: "Proceedings of a CEC-EPA-WHO International Symposium on Renal Advances in the Assessment of Health Effects of Environmental Pollution." pp 245. Commission of the European Communities Directorate General for Dissemination of Knowledge, Center for Information and Documentation CID, Luxembourg.

Kostial, K., Maljkovič, T. and Jugo, S. (1974). Arch. Toxicol. 31, 265.

Kostial, K. et al. (1975). In: "Proceedings of the 18th International Congress on Occupational Health." p 290.

Kovalskii, V.V., Yarovaya, G.E. and Shmavonyan, D.M. (1961). Z. Obsc. Biol. 22, 179 (In Russian; translation available through the Department of Environmental Hygiene, the Karolinska Institute, S-104 01 Stockholm 60, Sweden).

Levander, O.A. (1978). In: "Factors Influencing Metabolism and Toxicity of Metals." (G.F. Nordberg, ed) Op. cit.

Levander, O.A. and Baumann, C.A. (1966a).Toxicol. Appl. Pharmacol. 9, 98.

Levander, O.A. and Baumann, C.A. (1966b) Toxicol. Appl. Pharmacol. 9, 106.

Magos, L., Webb, M. and Butler, W.H. (1974). Brit. J. Exp. Pathol. 55, 589.

Mahaffey, K.R. and Fowler, B.A. (1977). Environ. Health Perspect. 19, 165.

Mazaleski, S.C., Coleman, R.L., Duncan, R.C. and Nau, C.A. (1970). Amer. Ind. Hyg. Assoc. J. 31, 183-188.

Moore, W. (1975). In: "Proceedings of a CEC-EPA-WHO International Symposium on Recent Advances in the Assessment of Health Effects of Environmental Pollution." pp 1171-1176. Commission of the European Communities Directorate General for Dissemination of Knowledge, Center for Information and Documentation CID, Luxembourg.

NAS (1972). "Airborne Lead in Perspective." Committee on Medical and Biological Effects of Environmental Pollution, National Academy of Sciences, National Research Council, Washington, D.C.

NAS (1978). "Zinc." Committee on Medical and Biological Effects of Environmental Pollution, National Academy of Sciences, National Research Council, Washington, D.C.

Nielsen-Kudsk, F. (1965). Acta Pharmacol. 23, 263.

Nordberg, G.F., ed (1976). "Effect and Dose-Response Relationships of Toxic Metals." Elsevier, Amsterdam.

Nordberg, G.F., ed (1978). "Factors Influencing Metabolism and Toxicity of Metals." Environ. Health Perspect. (In press).

Obermeyer, B.D. et al. (1971). Appl. Pharmacol. 20, 135.

Ohi, G. et al. (1976). Environ. Res. 12, 49.

Parizek, J. (1957). J. Endocrinol. 15, 56.

Parizek, J. (1972). In: "Nuclear Activation Techniques in the Life Sciences." I.A.E.A.-SM-157/82.

Parizek, J. (1976). In: "Effects and Dose-Response Relationships of Toxic Metals." (G.F. Nordberg, ed) pp 498-510. Elsevier, Amsterdam.

Parizek, J. and Ostadalova, I. (1967). Experientia 23, 142.

Parizek, J., Benes, I., Ostadalova, I., Babicky, A., Benes, J. and Lener, J. (1969). Physiol. Bohemoslov. 18, 95-103.

Parizek, J., Ostadalova, I., Kalouskova, J., Babicky, A. and Benes, J. (1971). In: "Newer Trace Elements in Nutrition." (W. Mertz and W.E. Cornatzer, eds)Pp 85-122. Marcel Dekker, New York.

Parizek, J., Kalouskova, J., Babicky, A., Benes, J. and Pavlik, L. (1974). In: "Trace Element Metabolism in Animals - 2." (W.G. Hoekstra, J.W. Suttie, H. Ganther and W. Mertz, eds) pp 119-131. University Park Press, Baltimore.

Petering, H.G. (1974). In: "Trace Element Metabolism in Animals - 2." (W.G. Hoekstra, J.W. Suttie, H. Ganther and W. Mertz, eds) p 311. University Park Press, Baltimore.

Petering, H.G., Johnson, M.A. and Stemmer, K.L. (1971). Arch. Environ. Health 23, 93.

Piscator, M. (1974). In: "Proceedings of a CEC-EPA-WHO International Symposium on Recent Advances in the Assessment of Health Effects of Environmental Pollution." pp 951. Commission of the European Communities Directorate General for Dissemination of Knowledge, Center for Information and Documentation CID, Luxembourg.

Piscator, M. and Lind, B. (1972). Arch. Environ. Health 24, 426.

Pond, W.G. and Walker, E.F. (1975) Proc. Soc. Exp. Biol. Med. 148,665.

Porter, K.G., McMaster, D. Elmes, M.E. and Love, A.H.G. (1977). Lancet 2, 774.

Potter, S. and Matrone, G. (1974). J. Nutr. 104, 638.

Schroeder, H.A. et al. (1966). J. Chron. Dis. 19, 1007.

Schütz, A. and Skerfving, S. (1975). Scand. J. Work Environ. Health 1, 54.

Silbergeld, E.K., Fales, J.T. and Goldberg, A.M. (1974). Nature 247,49.

Stonard, M.D. and Webb, M. (1976). Chem-Biol. Interac. 15, 349.

Stillings, B.R. et al. (1974). Toxicol. Appl. Pharmacol. 30, 243.

Szadkowski, D., Kohler, G. and Lehnert, G. (1969). Arztl. Forsch. 23, 271.

Task Group on Metal Interaction (1978). In: "Factors Influencing Metabolism and Toxicity of Metals." (G.F. Nordberg, ed) Op. cit.

Tsuchiya, K. and Iwao, G. (1978). In: "Factors Influencing Metabolism and Toxicity of Metals." (G.F. Nordberg, ed) Op. cit.

Underwood, E.J. (1977). "Trace Elements in Human and Animal Nutrition." Academic Press, New York.

Valberg, L.S., Sorbie, Y. and Hamilton, D.L. (1976). Amer. J. Physiol. 231, 462.

Welsh, S.O. (1976). Fed. Proc. 35, 761.

Welsh, S.O. and Soares, J.H., Jr. (1976). Nutr. Rep. Int. 13, 43.

Wester, P.O. (1974). Acta Med. Scand. 194, 505.

Whanger, P.D. (1976). In: "Selenium-tellurium in the Environment." p 234. Industrial Health Foundation, Pittsburgh.

Willoughby, R.A., MacDonald, E., McSherry, B.J. and Brown, G. (1972). Can. J. Comp. Med. 36, 348.

EPIDEMIOLOGICAL ASPECTS OF ASSESSMENT OF DOSE-RESPONSE AND DOSE-EFFECT RELATIONSHIPS

Tord Kjellström

1. The epidemiological approach

With the term epidemiology we mean the science of the occurrence of health and illness in human populations. In clinical medicine the individual and changes in the individual's health status constitute the focal point. In epidemiology the interest is extended to groups of individuals.

Epidemiological studies are essential for assessing dose-response or dose-effect relationships for toxic metals, because of the difference in the metabolism and toxicity of most metals between animals and humans. Results of animal experiments can seldom be used directly for quantitative conclusions about human effects and responses.

One of the main concepts of epidemiology is the population at risk. If, for instance, congenital malformations are under study they can only occur among newborn children and these newborn children could be defined as the population at risk. If spontaneous abortions are under study, the women who may become pregnant constitute the population at risk. In studies of metal toxicology the population at risk would further be limited to the group that was exposed to the metal. For example, in New Zealand there have been on the average about 4 cases of occupational lead poisoning reported every year during the 1970's (NZ Department of Health, annual reports). In a total population of 3 million (and 765,000 males in the age group 20-64, in whom most lead poisoning cases occur) this is not a common condition or a major public health problem. However, when we consider that the number of workers exposed to lead (the population at risk) was only 1740 on an average during the 1970's (NZ Department of Health, annual reports), the picture is different (Fig. 1). The incidence of reported lead poisoning in the population at risk was 2.3 cases (1000 person-years). This is similar to the incidence of hospitalization for cancer (2.4 cases/1000 person-years) and higher than the incidence of hospitalization for myocardial infarction and coronary heart disease (1.1 cases/1000 person-years) (NZDH, 1975).

The occurrence of disease or toxic effects is measured either as incidence, which is the number of newly developed cases during a defined time period, or as prevalence, which is the number of existing cases at a defined point of time. Incidence and prevalence are calculated as rates in the population at risk (e.g., number of cases per 1000 persons at risk). Depending on the type of disease or effect that is measured, the time unit used for incidence rates may vary. The most common for chronic effects is years. For acute effects days or even hours may be more appropriate. The time unit is included in the unit for incidence rate (e.g., cases/1000 person-years). The distinction between incidence and prevalence is important in studies of metal toxicology, because whenever the occurrence of effects is measured strictly in terms of prevalence or incidence, the population at risk, the time unit for effect measurements, etc., have to be more clearly defined than in studies where occurrence is measured only as proportion or percentage of an exposed group. There is room for a lot of improvement in this area. Even reputable journals like the Bulletin of the WHO publish articles on metal toxicology where the term incidence is used for prevalence data (Al-Mufti et al., 1976).

Incidence and prevalence rates are interdependent because naturally the number of existing cases depends on how many new cases occur. The duration of the disease and the time unit for incidence are the factors on which this interdependence is based. A low incidence may give a high prevalence if the duration of the disease is longer than the time unit for incidence. If the time unit for incidence is made very long, the calculation should be corrected for the increasing number of prevalent cases that can not get the effect again. The correction is made by calculating the cumulative incidence rate (Miettinen, 1977). This measure is particularly valuable in studies of the role of toxic metal exposure in the etiology of chronic diseases like cancer. Then, we are often interested in the cumulative incidence over a 30- or 40-year period and need to take into consideration increasing incidence rates with age. The cumulative incidence in the population at risk can for an individual be described as the risk (or probability) of getting the disease. The 40-year cumulative incidence rate for lead poisoning in New Zealand would be about 2/10,000 persons. The 40-year risk for an average lead exposed worker to get lead poisoning would be 0.01, or 1%.

In mortality and cancer-morbidity studies the life-table method for calculating individual risks has been used (Lemen et al., 1976; see section 5). From death registers and cancer registers the age-specific average incidence rates of dying from a particular disease or of getting cancer are calculated for a whole country or province. For each individual in the population at risk, these rates are used to estimate the risk of dying or getting cancer within the time period under study. The expected number of cases is calculated as the sum of the individual risks and this number is compared with the observed number of cases to calculate the risk ratio and the risk difference (Miettinen, 1977).

Epidemiological studies for assessing dose-effect and dose-response relationships of metal toxicity have the advantage that we are usually dealing with only one specific exposure - that to the metal compound. The problems lie in the measurement of dose. It is often necessary to estimate long-term doses due to long half-times of the metal compounds in the critical organ. This may be quite difficult (see section 4.2) because direct measurements are seldom possible. On the other hand, many of the toxic metal compounds are persistent in nature and this makes retrospective studies of e.g. museum specimens of food possible.

Measurements of effects may pose no problems particular to metal toxicity studies (see section 4.1) and the measurements of relatively high response rates can be made in studies of limited size. From the standpoint of prevention of effects it may be of greatest interest to measure very low response rates (e.g. 1 case/1000 persons). This necessitates large scale studies that are not always feasible because of cost or because the population at risk is not large enough. This is a feature of most environmental health epidemiology studies, but as will be discussed in the next section, so-called association studies make it possible to overcome this problem.

2. Observation versus association

Dose-effect and dose-response relationships for humans can be ascertained by epidemiological studies in basically two different ways. Observation studies involve measurement of average dose in groups within the population at risk and measurement of occurrence of effects and response in the same groups. Association studies involve the use of metabolic models, statistical distributions of the occurrence of the toxic metal in tissues or the daily intake, as well as estimates of critical concentrations in the critical organ to calculate the proportions of groups in the population at risk that are likely to have the effect (Figs. 2 and 3).

Observation studies are the most straightforward and they do not involve making assumptions about the metabolism of the metal compound, which may insert uncertainties in the dose-response evaluations. The disadvantage with this type of study is that it is often difficult to measure low response rates of effects directly when they are not routinely recorded. Exceptions are, for instance, deaths or cancers where these effects can often be ascertained from existing records.

There are many examples of dose-effect and dose-response evaluations of toxic metals using observation studies. Different degrees of effects of lead on hemoglobin synthesis (Roels et al., 1975) as well as different degrees of renal tubular damage by cadmium (Kjellström et al., 1977) have been studied in this way. A combined dose-effect and dose-response evaluation of neurological effects of methylmercury (Fig. 4) based on observations on 93 persons in Iraq (Bakir et al., 1973) is one of the most detailed studies of this type available, but still no observation data on response rates under 5% were recorded.

Association studies may be used as an extension of data from observation studies into parts of the dose-response relationship where no observation data exist or as a complete substitute for observation studies. The metabolic model is the key. The models may be one-compartmental as the one for methylmercury proposed by Berglund et al. (1971) or may take on one of many degrees of complexity. The model is a way of linking together data on metal intake, metal concentrations in different tissues and metal excretion. Data from groups with relatively low exposure levels can be used to quantify rates of transfer between compartments in the model. If it can be assumed that these rates are independent of the exposure levels (within the range of interest) critical organ concentrations under particular exposure conditions can be calculated and anticipated. Individual variation in dose within the population at risk as well as the individual variation of half-times of the metal compound in the main body compartments can be expressed as statistical distributions. In the case of cadmium, for instance, it has been shown that both daily intake and renal cortex cadmium concentrations follow log-normal distributions, which coincide remarkably well with calculations using a metabolic model (Fig. 5; Kjellström, 1977). The distribution in renal cortex can be used to calculate how large a proportion of the population with a particular average renal cortex cadmium concentration would have concentrations above a certain level.

The critical organ concentration is not defined as a threshold level above which pathological changes necessarily occur in the critical organ (Task Group on Metal Toxicity, 1976) in all individuals. For most metals it can be assumed that there is a considerable individual variation in the sensitivity of the critical organ. However, the best estimate of the metal concentration in the critical organ that is necessary to cause the critical effect would be the critical organ concentration.

By using metabolic models based on epidemiological data on metal intake, tissue distribution and excretion, as well as statistical distributions and estimates of critical organ concentration, the proportion of the population at risk who get the critical effect (=response rate) can be calculated. This can be done before human effects occur, which is an advantage with association studies. By definition observation studies can only be done when effects can actually be observed. Association studies have the disadvantage that the findings must always be judged as approximate due to the need for assumptions and due to limitations of the data collected. These drawbacks also apply when association studies are used to expand dose-response relationships documented by observation studies down to lower response rates, because slight errors in the middle part of the dose-response curve can become quite large at the extremes. Even so, such calculated dose-response relationships as those for methylmercury (Nordberg and Strangert, 1976) and for cadmium (Kjellström, 1977) can be of value to indicate what response rates can be expected at different doses. This can be used to decide on sample size in observation studies and to decide on provisional hygienic standards for primary prevention.

3. Study design

Epidemiological studies can be of three types. Descriptive studies provide data on the occurrence of exposures, dose or effects but give no direct comparison between dose and effects or dose and response. Studies of metal intake via food in a population or of the incidence of stomach cancer in different age and sex groups would be descriptive studies. Analytical studies put data on exposures or doses together with data on effects or responses in order to estimate the causative role for a particular exposure. Intervention studies involve manipulation (or intervention) of metal exposure in a randomized group in the population and follow-up of the influence on the occurrence of effects. Intervention studies are in a sense equivalent to human experiments and can only be used for the study of reversible effects that are not injurious to the persons exposed.

Analytical studies are the most common type of observation studies for assessment of dose-effect or dose-response relationships. Descriptive studies supply basic data for association studies.

The direction in which data are collected is of importance in analytical studies. The study can focus on persons who have the effect under study and compare them with a reference group without the effect, or it can focus on persons with the metal exposure and compare them with people without exposure. The first type of study is called a case-reference study (Table 1). The cases and non-cases are compared as to their metal exposures in the past, or as to the present metal dose based on analysis of indicator tissues. Higher doses in the group of cases would be interpreted as the metal being associated with this effect. Absolute occurrence can not be measured with a case-reference study alone, but the relative occurrence (risk ratio) between a high dose group and a low dose group can be calculated. If both the cases and the referents are stratified into groups according to metal dose, the risk ratio for each strata could be calculated and these ratios could be expressed as a dose-response relationship.

In order to measure the absolute incidence or prevalence rates in different dose groups, which must be done to establish complete dose-effect or dose-response relationships, a follow-up study would be more suitable. In this type of study an exposed group and a non-exposed group (referents) are selected. The occurrence of effects of the metal is measured in each group. The exposed group can be stratified into groups according to dose level and the measurement of incidence or prevalence of effects in each group forms the basis for dose-effect and dose-response relationships.

If there is a good indicator of dose, like for instance blood-lead for inorganic lead exposure (see Tsuchiya, 1977) or hair-mercury for methylmercury exposure (see Berlin, 1977), the data on dose and effects may be collected at the same time (cross-sectional study). Usually the data have to be collected longitudinally in time because of lack of good indicator material and because of changes of exposure with time which may not be reflected in the indicator material. The data can be collected either retrospectively or prospectively. Prospective data collection can of course be done in a more standardized, elaborate and controlled manner. When the half-time in the critical organ is very long, as for cadmium (see Friberg et al., 1977), retrospective exposure estimates are essential for arriving at results reasonably quickly.

One of the most important steps in an epidemiological study is the selection of the groups to be studied. In both descriptive and analytical studies it should be made certain that the people in the "exposed group" have really been exposed and belonged to the population at risk. Ideally the whole group that is and has been exposed should be studied or, if it is too large, a sample from this group should be randomly selected. In an industrial situation this means that all workers who ever were exposed in the factory under study should be included. An operational definition for "exposed" may be used such that an exposure duration limit is set (e.g. all workers who worked in the metal exposed area of the factory one year or longer). If only workers who are employed at the time of the study are included, those with the effect may be selectively excluded, because they had to stop working when they got the effect. In a dose-response study of cadmium-induced tubular proteinuria (Kjellström, 1977) it was shown that the possible underestimation of response due to selective exclusion may be considerable. Similar bias may occur in studies based on population screenings where only those healthy enough to come to the local school can participate. Selection bias may also be introduced in studies of industrial workers by the procedure of pre-employment selection of workers. Mainly healthy young men would be selected for the work that involves metal exposure and the results of such a study may not be applicable to the general population. This is called the "healthy worker effect" and it is a problem in e.g. retrospective follow-up studies of mortality (Kitagawa and Hansen, 1973).

The selection of the reference group in analytical studies is equally important as the selection of the exposed group. Selection bias caused by e.g. sick people at home not being able to participate, should be avoided. If any sampling of an exposed group takes place, the same criteria for sampling should be used for the reference group. Confounding factors can interfere with the analysis. A confounding factor is something that is associated with metal exposure and also associated with the effect; e.g. if the exposed group in a study of molybdenum and gout had a greater proportion of heavy drinkers than the reference group and heavy drinking was associated with gout, the drinking habit would be a confounding factor. Confounding can be controlled already at the stage of selection of target groups for the study by restriction of the target group to a sub-group of the population at risk. If drinking is a confounding factor, the study can be limited to non-drinking people only. If sex is a confounding factor, the study could be limited to women only, etc. Confounding can also be controlled for at the analysis stage of the study (see section 5).

4. Data collection

4.1 Measurement of effect and response

Effects can be classified into subjective symptoms, social or behavioral changes, biochemical or physiological changes, clinical disease, and death. Subjective symptoms can be e.g. the shortness of breath associated with beryllium exposure (Reeves, 1977) or the weakness, fatigue and anorexia associated with mercury vapor exposure (Berlin, 1977). In this group of effects we can also include subjective psychological and mood changes and annoyance reactions to dust, mucous membrane or skin irritation and smell. Data may be collected via standardized interviews or self-administered questionnaires. When interviews are carried out it is important to avoid observer bias caused e.g. by having different interviewers in the exposed group and the reference group. The way questions are asked may affect the answers. Ideally the interviews should be blind, which means that the interviewer should not know to which group a particular person belonged. A method for collection of subjective symptom data that has been used in air pollution effect studies is the panel method. Groups of people in an area with anticipated variations in exposure to air pollutants are asked to keep a diary of symptoms as they occur each day. During the same time period exposure levels are measured and any association between exposure and effects is analyzed. The panel method is only useful for studies of acute effects.

With social and behavioral effects we may mean family strife caused by behavioral changes in the affected person, decreased work efficiency, lagging behind in school work, absenteeism, and workmen's compensation or other insurance claims. The latter two are usually based on diagnosed clinical disease but this is not always the case and absenteeism may be an unspecific "annoyance" effect of metal exposures in the workplace. The data collection can be carried out in a similar way as for subjective symptoms or by using existing records of school work results, insurance claims, etc. Using this method it was found that high arsenic exposure during early life affected the children's school results 15 years later (Ohira and Aoyama, 1973). The effects mentioned here are quite unspecific and the risk of confounding may be greater than for other effects.

Critical and sub-critical effects of metal compounds are probably of the type biochemical and physiological changes but only for a few metals have they been established, e.g. decreased erythrocyte ALA-D activity after lead exposure (see Tsuchiya, 1977) and increased urinary β_2-microglobulin excretion after cadmium exposure (see Friberg et al., 1977). Histopathological changes may also be included in this type of effect. Measurement problems are related to finding the specific type of change caused by a metal compound. Animal studies can be of great value here, but species differences have been shown for instance for the type of proteins excreted in urine as an effect of cadmium poisoning. Another problem is to be able to take samples of the tissue where the effect occurs. The biochemical change we measure may be an indirect measure of the effect, like cadmium-induced urinary β_2-microglobulin excretion, or a direct measure, like lead-induced ALA-D inhibition.

When the effect is measured as changes in the concentrations of the metal compound normally occurring in tissue or in physiological variables, the dose-effect relationship can be evaluated from the quantitative increase in the variable with dose. For calculations of dose-response relationships it is necessary to arrive at an operational definition of what level of the variable constitutes an effect. The distribution of the variable in the reference group may be used to calculate a certain percentile as the "cut-off" level. When urinary concentrations of specific compounds are used for measuring effects it is necessary to correct the individual concentrations for variations in urinary dilutions. When compounds in whole blood are measured it may be necessary to correct for hematocrit.

Another problem with biochemical, physiological and histopathological changes as measures of effects is whether the changes measured should be integrated as adaptation to the metal or just physiological variations influenced by the metal. We do not have detailed knowledge about all the chemical steps in toxicity from the first changes to the severe clinical disease for any single metal. Any change that occurs in the critical organ that may be related to more severe effects in this organ should be considered at least as a sub-critical effect (Task Group on Metal Toxicity, 1976).

Effects of metals can also be measured as clinically diagnosed disease. Doctors' records, hospital records, data registers like a cancer register may be used as a source of data. Such records or registers may not be complete, however, and this may cause response to be underestimated. There may be factors that cause a biased selection of the people in the records or the register, like economic barriers or social class dependent attitudes to medical care. A complete clinical examination of the whole group under study may overcome such shortcomings of existing records. Cancer registers or congenital malformation registers are becoming more and more complete and accurate in industrialized countries where reporting systems are good and autopsy rates high. Cancers with a relatively short and characteristic course, like lung cancer or breast cancer, are more completely recorded than e.g. prostatic cancers which occur at older ages and may be overshadowed by other diseases that cause death. When this type of effect is measured it is just as necessary as in other cases to collect data for the exposed group and the reference group in an identical way. If a cancer register is used for calculating national reference rates for a "life-table study" (see section 5) all cancer cases in the exposed group should also be picked up via the cancer register. It must also be considered whether the fact that the exposed group (e.g. metal exposed factory workers) was exposed to carcinogenic metal compounds in itself makes it more likely for their cancer to be diagnosed and recorded in the cancer register.

Finally, the type of effect that is most easily defined, death, has been used in some studies as an indicator of long-term prognosis after metal exposure (Cooper and Gaffey, 1975). After very high exposures to metal compounds death can be one of the main effects (Bakir et al., 1973). Depending on the character of the recording system and autopsy rates, data on causes of death vary in quality. Mortality studies need long-term follow-up which poses problems of tracing the place of death of the target group and problems of identification. Case-reference studies of mortality involve finding data on exposures of people who can not be interviewed. The major difficulty in mortality studies is that individual dose data have to be linked up with the effect data since the effect itself cannot be measured.

When the target group is well defined and the effect has been measured accurately the measurement of response is straightforward: the proportion of the group who gets the effect in a specific time period (incidence response) or the proportion who has the effect at a specific point in time (prevalence response). Selection bias (see section 3) would be the most likely cause for errors in response measurement.

4.2 Measurement of dose

Ideally the metal dose should be measured in the tissue where the metal elicits its effect (Task Group on Metal Toxicity, 1976). In many cases this is not possible and indirect measurements of varying degrees of accuracy must be made instead (Fig. 6). The critical organ is often an internal organ that is not directly accessible for metal measurements. At autopsy, samples may be collected and analyzed and in some cases biopsy specimens have been used (e.g. renal biopsies from cadmium workers; see Axelsson and Piscator, 1971). Autopsy data may be compared with earlier data on effects (Nomiyama, 1973). For certain metals the recently developed in vivo neutron activation technique (Harvey et al., 1976) seems promising. No epidemiological studies using this technique have yet been published.

Indicator tissues like blood (Pb), urine (Cd), hair (methyl-Hg) or nails (As) can be used to estimate the dose in the critical organ. In order to translate indicator tissue concentrations into critical organ dose, metabolic models (see section 2) are useful. Feces amount of a metal is a good indicator of daily intake via ingestion when the gastrointestinal absorption is low. Comparison between cadmium body burden and daily fecal and urinary amounts of cadmium shows a good agreement between body burden and urine cadmium on a group basis after long-term low level exposure. Body burden and kidney burden have also been shown to be well correlated (Kjellström, 1977). Hair mercury is a good indicator of body burden of methylmercury (Friberg and Vostal, 1972) and because of the relatively even distribution of methylmercury in the body (see Berlin, 1977) it is also a good indicator of the amount of methylmercury in the brain. Hair may give us retrospective dose data because the metals stay in the hair as it grows out. It was shown in the methylmercury poisoning outbreak in Iraq (Bakir et al., 1973) that sectional analysis of hair could pinpoint the time when mercury consumption started and the time when it reached its maximum (Fig. 7).

The measurement of dose by analyzing indicator tissues or the critical organ itself involves chemical analysis of the metal compound in the tissue. The concentrations are usually very low, which makes accurate analysis difficult. The problems of chemical analysis have been highlighted by interlaboratory comparisons (Berlin et al., 1975) where differences up to two orders of magnitude were found. In order to make the data reliable they should always be supported by appropriate method studies, preferably including comparisons between two completely different types of analysis (like atomic absorption spectrophotometry and neutron activation analysis).

The dose may be estimated from exposure data if uptake kinetics and half-time in critical organ are known. The product of exposure level and exposure duration corrected for the ratio between duration and half-time and also for the absorption rate, etc., can give an estimate of dose. The calculation of dose should be based on the exposure that is relevant for uptake. Measurements of exposure to metals via air should include an estimate of particle size distribution and the metal concentration in each size fraction. The absorption of metals in the intestines can be affected by other elements in the diet (see e.g. Friberg et al., 1977; Tsuchiya, 1977). Therefore, additional information on diet content would be necessary to calculate dose from exposure levels.

Most metals, because they are elements, occur naturally in air, food and drinking water. Whatever particular exposure conditions are under study we usually have to consider the total exposure by adding all possible uptakes. Skin exposure may be significant in occupational environments.

Smoking can increase exposure both through the natural content of metals in cigarettes and pipe tobacco and through external contamination of the smoking gear from hands, overalls or the workroom air (Piscator et al., 1976). A smoker may of course be sensitive to airborne metal exposures in other ways. Smoking is associated with chronic bronchitis and emphysema and it slows down lung clearance (Albert et al., 1969).

We can assume that for certain effects of certain metals the dose may not be a function of exposure level times the exposure duration, but rather a function of the frequency of peak exposures above a certain level (impulse exposures). When carcinogenic effects of metals are studied, the dose is calculated in three different ways depending on the assumed mechanisms for carcinogenicity (Task Group on Air Pollution and Cancer, 1977).

5. Data analysis

This is the stage in an epidemiological study where the data are arranged so that conclusions can be drawn. In descriptive studies the conclusion would be in the form of a description of the occurrence of a variable in the population group studied and in an analytical or intervention study it would be in the form of rejecting or accepting a hypothesis about an association between an exposure and an effect. If the study had been designed for ascertaining a dose-effect or dose-response relationship, the conclusion may be that this was or was not found. In drawing these conclusions a number of standard statistical techniques are used.

Quantitative dose data can be expressed as distributions (Fig. 8) of individual doses. From the distribution it can be calculated how large a proportion of a population can be expected to have a dose above levels associated with certain effects. We can also express the dose as population dose, which means the sum of all individual doses in a population. This is the approach often employed in radiation protection and basically it assumes a linear dose-response relationship down to very low doses. A certain population dose is assumed to be associated with a certain number of cases with effect. If the dose data are in the form of environmental measurements of emissions, it may also be relevant to estimate the dose commitment. This is the estimated total future population dose that will arise from the emissions.

Quantitative effect data can also be expressed as distributions. A difference between the distributions is most easily ascertained with the Kolmogorov-Smirnoffs test.

When the effects are measured as the number of cases of a particular disease (e.g. primary liver cancer), and incidence data based on a total population are available, the life-table technique can be used to estimate incidence ratios between exposed group and total population. A starting point in time for the calculation is defined. This may be when the persons started exposure, 20 years after they started exposure, or some other particular date (like the day when the cancer register became operational). For each exposed individual the age of the starting point and the calendar year of the starting point are used to find, in the register data, the incidence of the disease in the total population for that age group at that time. The incidence value is interpreted as that person's risk of developing the disease that particular year. By adding up all the individual risks for each year after the starting point, the yearly expected number of cases is calculated. This is compared with the actual observed number to yield the incidence ratio (observed cases/expected cases).

In case-reference studies a Venn-diagram may be useful for figuring out the incidence ratios (Fig. 9). When more than one exposure variable are evaluated at the same time (e.g. cadmium and smoking exposure and local effects on the lung) a Venn-diagram may show clearly what type of additional data is needed when some data are already available.

The shape of the dose-response curve is often sigmoidal like a cumulative normal distribution. Such a curve can be transformed to a straight line by probit-transformation. When the effect is not specific for the metal exposure but also occurs at a low level in a non-exposed population, the response rates should be adjusted before the probit-transformation. This and other aspects of probit analysis of dose-response data for toxic metals have been discussed by Kjellström (1976). In the case of cancer, it has now been proposed (Task Group on Air Pollution and Cancer, 1977) that at low doses the dose-response curve can be approximated by a straight line. The implication is that there is no safe threshold for carcinogenicity. This has great influence on the assessment of hygienic standards to protect people from cancer-inducing metals. If the cancer-risk at dose A is 1/1000 then the risk would be 1/100,000 at dose A/100, which may be a common dose in large population groups, and public health protection measures may be needed.

REFERENCES

Albert, R.E., Lippman, M. and Briscoe, W. (1969). Arch. Environ. Health 18, 738-755.

Al-Mufti, A.W., Copplestone, J.F., Kazantzis, G., et al. (1976). Bull. WHO 53, (suppl.), 23-57.

Axelsson, B. and Piscator, M. (1971). Unpublished data. In: "Cadmium in the Environment." (L. Friberg, M. Piscator and G. Nordberg) 1st edition. CRC Press, Cleveland.

Bakir, F., Damluji, S.F., Amin-Zaki, L., Murtadha, M., Khalidi, A., Al-Rawi, N.Y., Tikriti, S., Dhahir, H.I., Clarkson, T.W., Smith, J.C. and Doherty, R.A. (1973). Science 181, 230-241.

Berglund, F., Berlin, M., Birke, G., Cederlöf, R., von Euler, U., Friberg, L., Holmstedt, B., Jonsson, E., Lüning, K.C., Ramel, C., Skerfving, S., Swensson, Å. and Tejning, S., (1971). Nord. Hyg. Tidskr., Suppl. 4.

Berlin, M. (1977). In:"Toxicology of Metals." Volume II, pp 301-344. Environmental Health Research Series, 600/1-77-022, Environmental Protection Agency, Research Triangle Park.

Berlin, A., Lauwerys, R., Buchet, J.P., Roels, H., Del Castilho, P. and Smeets, J. (1975). In: "Proceedings of a CEC-EPA-WHO International Symposium on Recent Advances in the Assessment of Health Effects of Environmental Pollution." pp 2185-2213. Commission of the European Communities Directorate General for Dissemination of Knowledge, Center for Information and Documentation CID, Luxembourg.

Cooper, W.C. and Gaffey, W.R. (1975). J. Occup. Med. 17, 100-107.

Friberg, L. and Vostal, J. (1972). "Mercury in the Environment." CRC Press, Cleveland.

Friberg, L., Nordberg, G. and Piscator, M. (1977). In: "Toxicology of Metals." Volume II, pp 124-163. Environmental Health Research Series, 600/1-77-022, Environmental Protection Agency, Research Triangle Park.

Harvey, T.C., McLellan, J.S., Thomas, J.J., et al. (1976). 2nd East Kilbride Conference on Progress and problems of in vivo activation analysis. Scottish University Research and Reactor Centre, Glasgow.

Kitagawa, G.M. and Hansen, P.M. (1973). "Differential Mortality in the United States." Harvard University Press, Boston.

Kjellström, T. (1976). In: Nordberg, G.F., ed. "Effects and Dose-response Relationships of Toxic Metals." pp. 147-156. Elsevier, Amsterdam.

Kjellström, T. (1977). Accumulation and renal effects of cadmium in man. A dose-response study. Diss., Karolinska Institute, Stockholm.

Kjellström, T., Evrin, P.-E. and Rahnster, B. (1977). Environ. Res. 13, 303-317.

Lemen, R.A., Lee, J.S., Wagoner, J.K and Blejer, H.P. (1976). Ann. NY Acad. Sci. 271, 273-279.

Miettinen, O.S. (1977). "Principles of Epidemiologic Research." Duxburg Press, Massachusetts (In press).

Nomiyama, K. (1973). Jap. J. Hyg. 28, 45 (In Japanese).

Nordberg, G.F. and Strangert, P. (1976). In: Nordberg, G.F., ed., "Effects and Dose-response Relationship of Toxic Metals." pp. 273-282. Elsevier, Amsterdam.

NZDH (1975). Trends in health and health care. New Zealand Department of Health.

Ohira, M. and Aoyama, H. (1973). Jap. J. Hyg. 27, 500-531 (In Japanese).

Piscator, M., Kjellström, T. and Lind, B. (1976). Lancet 2, 587.

Reeves, A. (1977). In: "Toxicology of Metals." Volume II, pp 85-109. Environmental Health Research Series, 600/1-77-022, Environmental Protection Agency, Research Triangle Park.

Roels, H.A., Lauwerys, R.R., Buchet, J.P. and Vrelust, M.T. (1975). Int. Arch. Arbeitsmed. 34, 97-108.

Task Group on Air Pollution and Cancer - Risk Assessment Methodology and Epidemiological Evidence. (1978). Report from an International Symposium at the Karolinska Institute, Stockholm, 1977. Environ. Health Perspect. (In press, 1978).

Task Group on Metal Toxicity (1976). In: Nordberg, G.F., ed., "Effects and Dose-response Relationships of Toxic Metals." pp. 7-114. Elsevier, Amsterdam.

Tsuchiya, K. (1977). In: "Toxicology of Metals." Volume II, pp 342-400. Environmental Health Research Series, 600/1-77-022, Environmental Protection Agency, Research Triangle Park.

Table 1. Classification of epidemiological studies

Type: Descriptive (just listing observations)
Analytical (testing associations between exposure and effects)
Intervention (the "experiment")

Direction: Case-reference (start from cases with effect and probe into their exposures)
Follow-up (start from exposed people and see if they get the effect)

Time: Cross-sectional (all data collected referring to one point in time)
Longitudinal:
    Retrospective (data collected regarding the present and the past)
    Prospective (data collected regarding the present and the future)

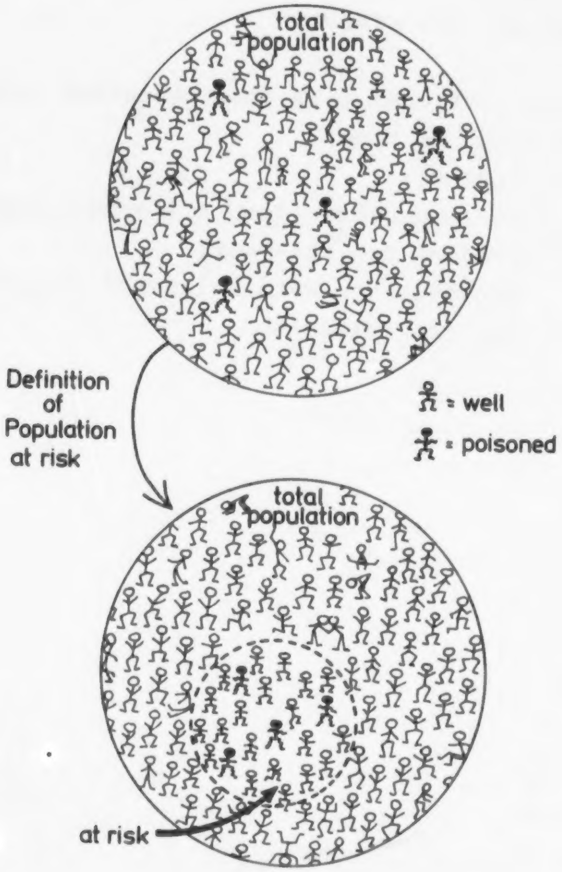

total population
Definition of Population at risk
= well
= poisoned
total population
at risk

Figure 1. Diagram indicating the relevance of defining a population at risk. Without such a definition the response estimate will be "diluted".

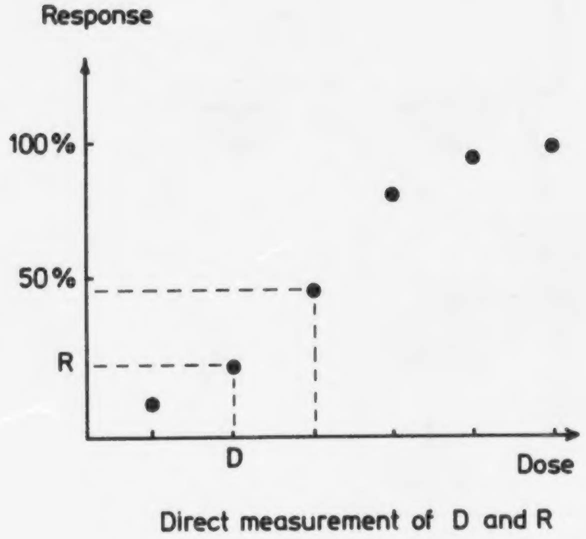


Figure 2. Observation studies. The target population is stratified into groups according to dose. The mean dose and the response are measured for each strata giving one point each on the dose-response curve.

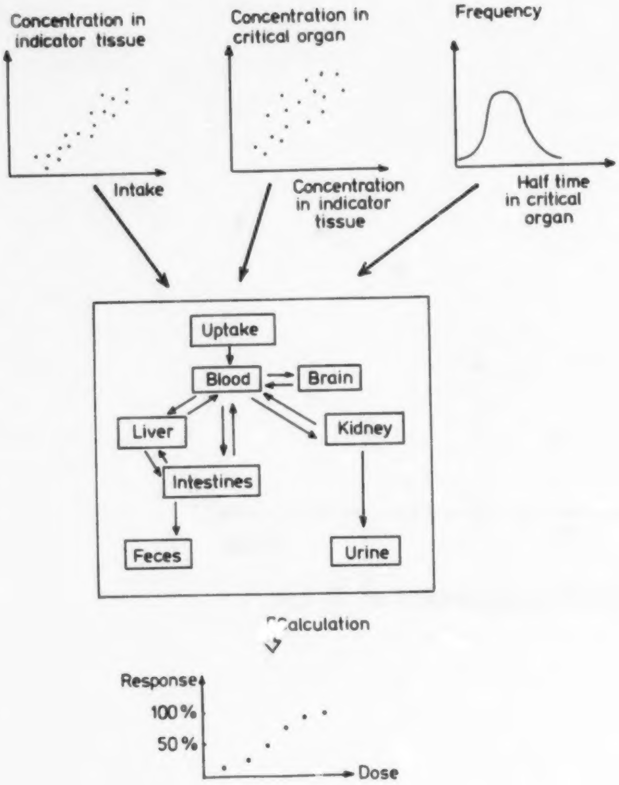

Concentration in indicator tissue
Intake
Concentration in critical organ
Concentration in indicator tissue
Frequency
Half time in critical organ
Uptake
Blood
Brain
Liver
Kidney
Intestines
Feces
Urine
Calculation
Response
100 %
50 %
Dose

Figure 3. Association studies. Epidemiological data on human metabolism of the metal and distribution of half-times, etc., are used to establish a metabolic model. The model calculates the proportion of a group with a particular dose that would have metal concentrations in critical organs above an estimated critical concentration. This is used as an estimate of response rate in each dose group.

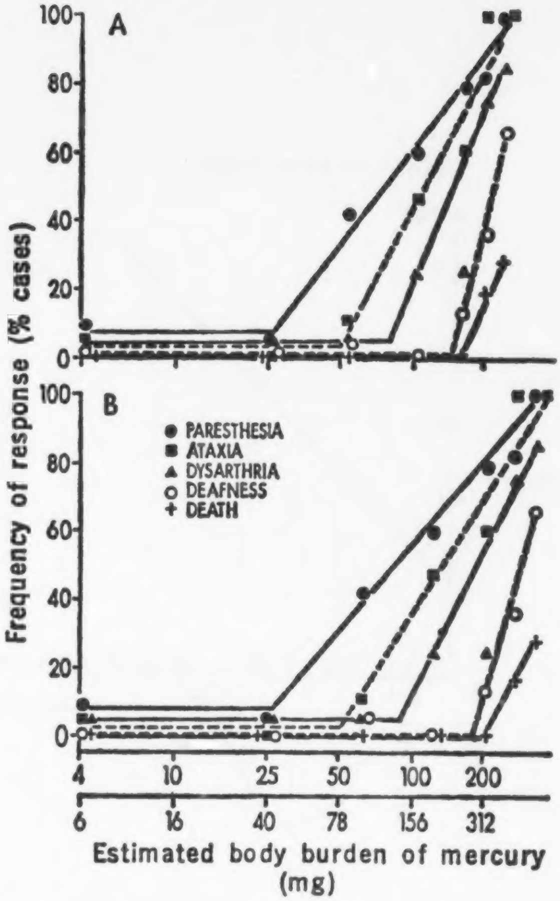


Figure 4. Dose-response relationship for different symptoms and signs in the poisoning incident in Iraq. Dose estimates from blood mercury levels.
A = at the time of onset of symptoms;
B = at the time of cessation of exposure
(from Bakir et al., 1973).

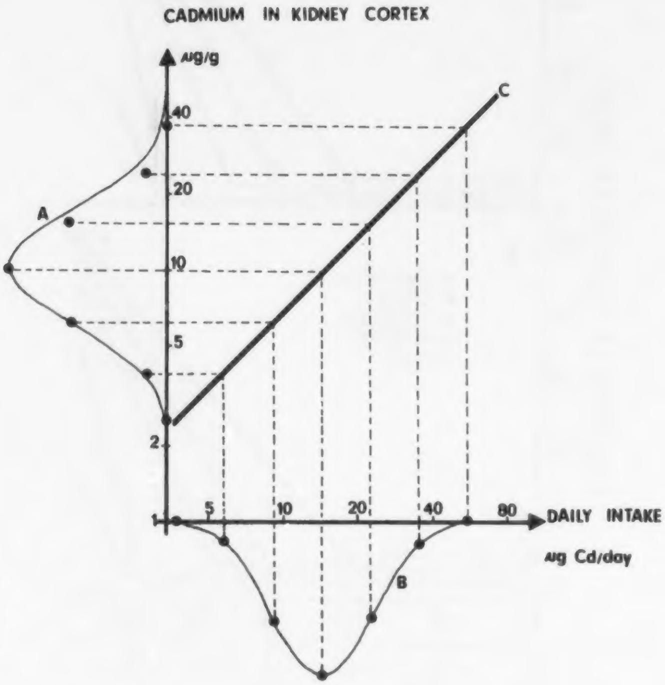


Figure 5. Relationship between distribution of daily cadmium intake (B) and distribution of cadmium concentrations in kidney cortex in the age group 30-59 in Sweden (A). ● = calculated relationship from a metabolic model (C) (from Kjellström, 1977).

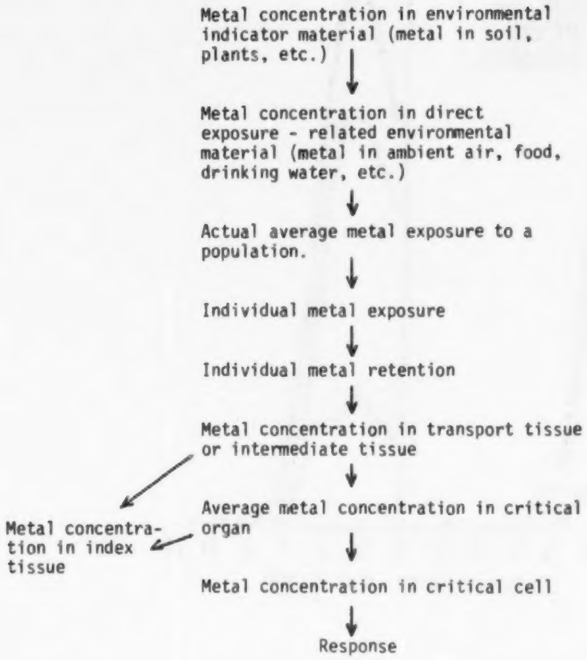

Metal concentration in environmental indicator material (metal in soil, plants, etc.)
Metal concentration in direct exposure - related environmental material (metal in ambient air, food, drinking water, etc.)
Actual average metal exposure to a population.
Individual metal exposure
Individual metal retention
Metal concentration in transport tissue or intermediate tissue
Average metal concentration in critical organ
Metal concentration in index tissue
Metal concentration in critical cell
Response

Figure 6. Sequential relationship between dose variables and a specific response (from Kjellström, 1976).

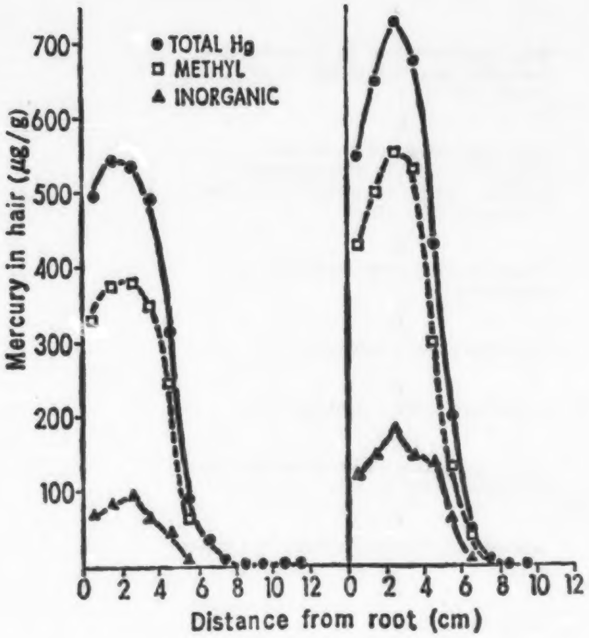


Figure 7. Concentrations of mercury (methylmercury, inorganic mercury and total) in hair sections from two patients with methylmercury poisoning in Iraq (from Bakir et al., 1973).

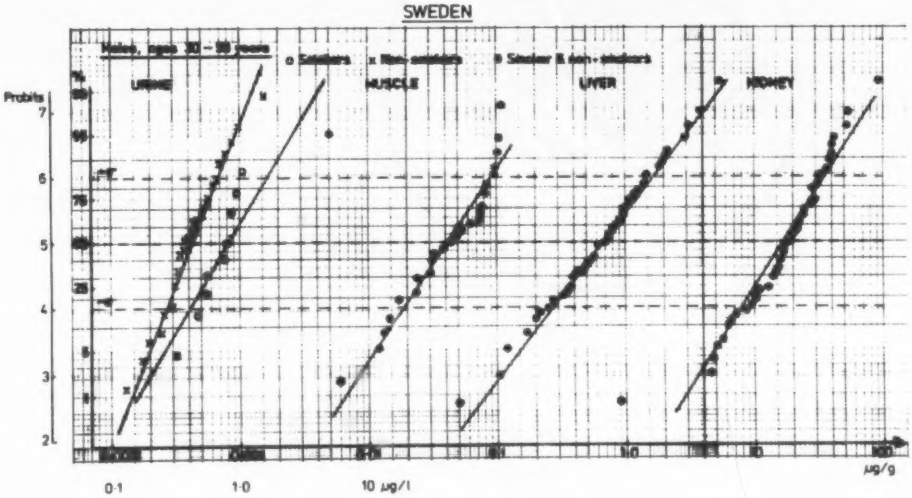


Figure 8. Distribution of cadmium doses measured as urine, muscle, liver or kidney cortex concentrations (from Kjellström, to be published).

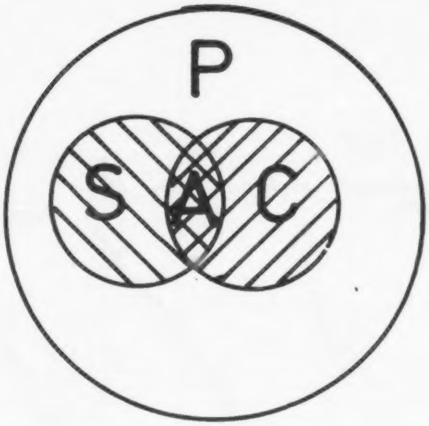


Figure 9. Venn-diagram of data from a case reference study, e.g. P = population, S = smokers, C = cancer cases, A = smoking cancer cases. Data from the study = S/P and A/C. The risk ratio for cancer among smokers as compared to whole population is $\frac{A/S}{C/P} = \frac{A/C}{S/P}$.

GENERAL ASPECTS OF THE PREVENTION OF METAL POISONING

Sven Hernberg

1. Goals: Ideals and practical restrictions

There are many definitions of poisoning, the narrowest requiring the presence of overt disease, and the widest including any functional deviation from normality. From the preventive point of view the concept of poisoning should be regarded in its widest sense. The ultimate goal should be an environment in which exposures to toxic metals have been reduced to levels at which no functional impairment is caused, either in the present population or in future generations.

In practice the ultimate goal is far from reached. The main obstacles are economical and technological, for example, the removal of metals already dispersed in the environment, the design of completely safe work processes or the efficient sanitation of old plants. Hence operative goals must often differ from the ideal, and the preventive strategy chosen is not always the most efficient.

In the prophylaxis of metal poisoning, the assumption is often made that a no-effect level exists for the toxic action of metals. This assumption implies that some exposure can be tolerated, and it has resulted in the endorsement of several different types of health criteria. Some standards are not determined by health aspects only, but also from the beginning take into consideration economical and technological feasibility. Criteria for occupational exposure sometimes even accept some deterioration from normal health. Some types of effects, such as the lead-induced inhibition of erythrocyte delta-aminolevulinic acid dehydratase, are presently thought to be acceptable, even though they occur in a large proportion of the persons exposed. Others are accepted if only a small portion exhibit them. For example, in the documentation of the ACGIH Threshold Limit Values it is stated that "nearly" all workers may be repeatedly exposed without adverse effect (ACGIH, 1974; also the chapter on standards and criteria in this volume).

The acceptability or non-acceptability of functional impairment is a delicate matter indeed, and the criteria vary according to who defines them and to the circumstances under which they should be applied. A good illustration is the well-known dualism that exists between most standards for the general as opposed to the work environment. Efforts are being made in several countries, at considerable cost, to maintain the upper range of lead in blood at 40 or even 30 µg/100 ml for the general population, mostly by banning the addition of lead to gasoline. Still, it is persistently claimed that industrial workers and their unborn children can easily tolerate levels of 70 or even 80 µg/100 ml.

2. General principles of prevention

In all prevention there are some common principles. First, the hazard must be recognized and the routes and modes of exposure determined. This knowledge may be readily available or it may require a vast amount of research. The recognition of the dangers of discharging mercury into the water system is a good example of a difficult problem. It was in Japan, where after several years of confusion, the so-called Minamata disease was recognized as being due to the ingestion of seafood contaminated by methylmercury (Japanese Ministry of Health and Welfare, 1967). Since discharges often include only inorganic mercury, the next step was to demonstrate that metallic mercury is methylated in an aquatic environment (Jensen and Jernelöv, 1969). It was also discovered that methylmercury is enriched in food chains (Friberg and Vostal, 1972). Because of these findings large surveys of the content of methylmercury in fish were initiated in Sweden, and later in other countries. Concomitantly, estimations of the highest safe daily intake were agreed upon. All of this activity made it possible to localize the hazard and to take administrative action. The most important measures initiated were the strict control of the discharge of mercury into water systems and a ban on the sale of fish of prey from contaminated waters, together with recommendations for the maximal weekly intake of fish.

In many cases prevention is simple as soon as the hazard is recognized. For example, leaded gasoline is often (mis)used as a solvent, especially in small industries. Prevention of lead poisoning is extremely simple: substitution with lead-free gasoline or some other solvent.

Some general guidelines that can help identify occupational hazards will be given later.

The second principle of prevention lies, of course, in the elimination of the hazard, if possible. Generally speaking, the following procedures should be undertaken:

- cessation of unnecessary use of the toxic metal,
- substitution with less toxic agents,
- prevention of the spread of dust and fumes,
- removal of the metal already spread, and
- reduction of personal contact with the metal.

As long as exposure levels are potentially hazardous, or may become so, monitoring the environment and the exposed population (or some indicator group), the third principle, is warranted.

Most, if not all, preventive efforts fail if the professional staff is not well trained. Training, the fourth principle, should not be restricted to health personnel and engineers. Both the factory workers and the general public should have basic knowledge of how to protect themselves, and should be made aware of the importance of control measures. Securing both is an important task for those responsible for the health care of a plant or a community. For example, if lead oxide dust is generated in a restricted area of a factory and its elimination is based on effective local exhaust ventilation, the workers should know how to prevent it from spreading to other parts of the plant. Similarly, in order for house-to-house campaigns aimed at eliminating childhood lead poisoning due to the ingestion of flaking paint to succeed, health officers must win the cooperation of the families concerned.

Most programs, at least large-scale ones, should be backed by some form of authority, this being the fifth principle. Usually such authority is gained through legislation, sometimes by agreements between, e.g. labor and management. Before such measures are taken, there may be a need to create political pressure. Those engaged in research must not withdraw into an ivory tower; on the contrary, their task is to provide politicians with unbiased data and opinions based on scientific judgement. They should also offer the public correct facts and fight against any form of exaggeration and misrepresentation.

3. The work environment

3.1 General considerations

In the work environment, the main route of exposure is through inhalation, and hence preventive measures should be concentrated upon reducing the levels of airborne fumes and dusts. In this respect the general principles of industrial hygiene apply. Since there are several good textbooks on this topic (e.g. Patty, 1962; Olishifski and McElroy, 1971), only a brief recapitulation, with emphasis on the aspects typical of metals, is given here.

Recognition of hazards. Before any action can be taken, the hazard must be recognized. Many countries require the labeling of toxic products and a declaration of their composition. In some instances this regulation may help. No simple rule of thumb exists for a general classification of occupations, workplaces or work phases as "dangerous" and "less dangerous". On the contrary, the hazard is determined by the many factors that influence the concentrations of respirable metal dust or fumes in the air. First, the process itself is important. All high processing temperatures, extensive fume, dust or aerosol formation, and primitive work methods increase the risk; and low temperatures, low-level dust formation, automation, and encapsulation promote safety. Secondly, efficient local and general ventilation and the proper prevention of the spread of dust reduce the risk while a lack of such measures increases it. Thirdly, the general level of workplace hygiene is important. Considerations of this type mean that workplaces must be looked at individually as far as risk evaluation is concerned. If any general rule could be given, it would be that an accumulation of negative factors is more usual in small, primitive plants than in large, modern ones.

Elimination of unnecessary use. In some instances the production of toxic compounds or the use of the toxic metal itself in a process is unnecessary. Some brass castings should not be allowed at all in primitive foundries, since automation eliminates the risk. When planning new chlorine plants, the diaphragm method should be considered since it does not call for any mercury at all.

Substitution. Because metals are fundamental to our civilization, substitution of other materials for metals cannot be widely applied. This has been possible as far as mercury nitrate, which was used for more than two centuries in making felt for hats, is concerned. This procedure resulted in outbreaks of severe poisoning and in such expressions as "mad as a hatter". Mercury nitrate has now been replaced by less toxic chemicals in most countries, although some exceptions are still said to occur (Hamilton and Hardy, 1974). The use of lead arsenate as an insecticide has largely been abandoned. Red lead or litharge (Pb_3O_4), once the most important anti-corrosive paint in shipbuilding, is increasingly being replaced by less toxic paints (Ziegfeld, 1964), and white lead (carbonate) has been avoided, sometimes due to laws, in indoor painting for decades. In Finland, legislation prohibiting such use was passed as early as 1929 (Hernberg, 1973). In the pottery industry, the introduction of insoluble fritted lead polysilicates as glazes has somewhat diminished the exposure.

On the other hand, substituting the lead sheet of cables with a plastic covering has increased the risks involved in their manufacture, since the new material (PVC plastic) is produced with lead stearate used as a stabilizer. Mixing this substance into the raw material has proven to be more hazardous than smelting metallic lead (Tola et al., 1976). In the vast majority of operations, metals or their compounds are not easily replaced, and other methods of prevention must be applied.

Technical control measures. The technical control of metal exposure follows the principles applied in industrial hygiene in general. Accordingly success is usually the greatest when the technical and medical knowledge we have today can be used in the planning stages. Old plants may be difficult to remodel satisfactorily. If possible, all hazardous types of exposure should be concentrated in special areas where control processes can be effectively utilized. Encapsulation and other isolation methods prevent toxic dust and fumes from being spread about while mechanization and automation reduce the number of workers exposed. Furthermore, special well-ventilated control rooms can be built within the plant. Surfaces should be plane and smooth in order to prevent dust from attaching to walls and floors. Nooks and corners should be avoided. For example, in the case of metallic mercury, the worst problem is evaporation from spilled droplets. Their removal is facilitated by smooth, unabsorbing surfaces which slant towards the center of the floor. Secondary lead smelting is such a hazardous process that priority should be given to automation of transportation and oven filling phases. More reduction of the levels of lead in the working environment can be accomplished if the ovens can be emptied in the open air. The preparation of all metal pigments, very hazardous when done by hand, is far less so in closed, automatic systems.

Local exhaust ventilation. One of the best means for controlling dust or fumes that cannot otherwise be prevented from escaping into the air is a local exhaust system. The air must then be filtered or the metals otherwise removed so that the general environment will not be polluted. There is a persistent belief that the exhaust should be from below since metal dust particles in general are heavy. However, the respirable fraction is made up of small particles capable of remaining airborne for long periods and behaving like a gas cloud. Hence, to be efficient, the exhaust should be placed very close to the source of pollution. The system for removal of the polluted air must be constructed so as not to draw the air into the breathing zone of the worker on its way to the exhaust.

General ventilation. A good general ventilation is an adjuvant to a local exhaust system. Since the general systems involve a much higher cost, e.g., in energy consumption, the emphasis should be put on the local ones. One important exception is the elimination of metallic mercury vapors. Because the droplets are usually spread over the floor and because evaporation will be reduced by lowering the work room temperature, general ventilation should be well developed. It should be stressed that open windows and doors as well as changes in the work process may thwart an otherwise effective ventilation system. Self-made "improvements" and extensions may be deleterious, and the ventilation, filtration and precipitation equipment may deteriorate.

Other methods of elimination. Since most metals occur in dust form or precipitate as small particles, exposure can be reduced by keeping the floor permanently moistened, and by frequent vacuum-cleaning. Sweeping should be avoided, as this operation returns settled dust to the workroom air. If sweeping cannot be avoided, oiled sawdust should first be spread on the floor to bind the dust. The use of compressed air to blow dust from floors, walls and ledges should be prohibited. In principle, all cleaning or other operations that may cause settled dust to whirl about should be performed outside the work shift by well-protected, special workers.

When moistening the floor, the water should not come in contact with molten metal, since violent explosions would occur. Operations involving the welding, flame-cutting or burning of metals coated with paint of unknown composition should not be allowed before samples have been analyzed for at least lead, cadmium and chromates, or the composition otherwise elucidated. If such metal pigments are present, the burning is extremely dangerous and should be performed under powerful local exhaust and general ventilation, with safety equipment, and, if possible, outside the normal work shift. Metals coated with metallic cadmium should be treated in the same way. Even less toxic metals, such as zinc and copper, cause metal fume fever, and welding and burning such coatings demand the same protective measures.

Housekeeping. Poor housekeeping can render even the best technical control system ineffective. Education and supervision should be detailed, efficient and followed up and motivation should be kept at a high level. Smoking can never be allowed in the presence of metal dust. Clothing made of synthetic fibers retains less dust than do cotton overalls. Pockets should be as few as possible, especially when metallic mercury is present in the work room. Work clothing should be vacuumed before removal and changed at least twice a week. Work and street clothing should be stored in different lockers to reduce the exposure of the worker himself and to prevent him from exposing his family. Because of the danger of food becoming contaminated from the environment or from dusty hands or clothing, it should not be prepared, dispensed or eaten in areas where metal dust occurs. Clean dining rooms should obviously be available. Personal hygiene should be facilitated by providing sufficient washrooms, and requiring that they be used. This is important also from the point of view of reducing family exposure.

The worker should spend as little time as possible in areas with unusually high concentrations of metal dust or fumes. These areas include the vicinity of smelting furnaces, welding places, pasting machines in storage battery factories, etc. When engaged in welding or burning outdoors, the worker should always place himself upwind, and other workers should not be allowed to work downwind of the welding or burning operation.

Reduction of worker contact with toxic metals. If the large-scale engineering methods such as encapsulation fail to ensure enough safety, personal protective equipment, mostly respirators, must be used, but only as a last resort. The only situations where respirators can be considered a primary choice are in emergencies or in occasional, brief exposures above the threshold limit value. Many types of filter respirators are on the market, but not all of them are adequate as far as meeting requirements outlined in for example the American National Standard for Respiratory Protection Z 88.2-1969 is concerned. Metal fumes require more efficient respirators than dust; if both are present, those for fumes are recommended. In certain operations where exposure is very high, for example, when ships covered with thick layers of red lead are scrapped by flame-cutting, the only acceptable type of protection is the air-purifying positive-pressure respirator (NIOSH, 1972). In addition to other preventive measures, reduction of exposure time either by shortened daily exposure time or by worker rotation every few weeks or months is necessary e.g. often in shipbreaking and secondary lead smelting, and sometimes in chlorine alkali plants (where the problem is exposure to metallic mercury vapors). For metals, such as cadmium, which accumulate to a high degree in the body a shortened lifetime exposure may even be considered, whereby workers who have accumulated large amounts of cadmium should be taken from exposure for good, regardless of whether or not they exhibit symptoms.

3.2 Monitoring of the work environment

Measurements of concentrations of metals in the work environment are mandatory for a preventive program in order to:

- localize high exposure areas
- estimate individual exposure (especially when no satisfactory biological monitoring method exists)
- detect engineering failures (leaks, insufficient ventilation, etc.)
- detect the effects of changes in processes or work methods
- document the impact of preventive measures
- ensure that safety standards are being met.

Sampling strategy. Concentrations close to a machine or process are maximal. They may vary considerably over a distance of a few meters or even centimeters, especially when the sources of contamination are few such as in the case of a smelting furnace. For most purposes sampling should be performed in regular work areas and from the worker's breathing zone.

The number of air samples needed depends upon the purpose of the measurement. An overall picture is obtained from sampling carried out on a long-term basis and from different sites. When individual exposure is to be studied, workers who are representative for each area, operation and other special conditions should be chosen. Especially those with predicted high exposure should be considered. The alternative is to use some random sampling procedure, especially when there is little advance knowledge about the exposure.

Air sampling may be performed at regular intervals, or randomly over time. Regular intervals must not coincide with other regular cycles of events that may cause systematic variations in the measured concentrations.

Short-term samples are preferred for intermittent exposure, as for example in welding operations. Long-term samples (7 to 8 hours) are the most relevant when one primarily wants to ensure that the metal concentrations on the average are below the threshold limit value. Because long-term particulate sampling often has to be restricted to about 10 samples or less for practical reasons (e.g. 8 hours/one sample), their average should be well below the threshold limit value if a safety margin is to be ensured for variations.

Personal monitoring is of course the method of choice when individual exposure is to be estimated as far as air sampling is concerned. The samples should be taken from the breathing zone, no farther than 40 cm from the nose. It may even be taken from within a welding helmet. The sampler is usually attached to the worker's shoulder. No obstruction can be allowed between the air sampler inlet and the worker's face, and the equipment must not disturb his work, e.g. by blocking his sight. Compared to biological samples, personal monitoring may give a much less correct picture of exposure unless strict precautions and standardization are enforced (Williams et al., 1968; Chatterjee et al., 1969; Williams et al., 1969; Williams, 1975). For example, the angle of the inlet is important, as well as its distance from the nose. Furthermore, personal sampling does not allow for differences in physical activity during exposure, personal hygiene, use of respirators, or in other exposure (from food, water, etc.). When the metal is cumulative, exposure during one day is not very informative from the point of view of assessing the health risk. Hence, biological monitoring is always preferable whenever reliable methods exist. Such is definitely the case for lead, partly the case for mercury and perhaps for cadmium and arsenic. For other metals, knowledge on the relationship between the concentrations in ambient air and those in biological media is still insufficient.

Sampling technique. Metals usually appear in the workroom air as fumes and solid particles, sometimes as vapor (metallic mercury) or liquid aerosols (chromates above the electroplating bath). Fumes are formed when molten metal evaporates or when metal or metal compounds are burnt. The dust particles are either derived from condensed fumes or from metal compound dust that has whirled up. Although the particle size distribution varies, the respirable dust is composed of small particles that can stay in the air for many hours. Larger particles settle but may be whirled up again.

During sampling a measured volume of air is drawn through a filter or impinger device, with the use of an electrostatic or thermal precipitator, a cyclone, or some other instrument which collects particles. Meters for direct-reading of total dust or mercury vapor concentrations are also available.

The volume of the air sample may vary from a few liters to several cubic meters, depending on the concentration and the sampling equipment. It must contain enough particles to be accurately weighed or analyzed. Often the concentrations, especially those around or below the threshold limit value, are so low that a large air volume and high flow rate are needed before enough metal particles can be collected during a reasonable period of time. The sample size should be large enough so that accurate estimations of concentrations within the magnitude of about a tenth of the threshold limit value can be made.

Analysis. The concentration of the particulates in the air is calculated from the weight of the particulates collected per unit volume of the air sample, determined by direct weighing and/or chemical analysis. The particulates in a known portion of the sample are counted for the determination of the number of particles collected. This procedure is accomplished by microscopy or by automatic particle counters. Since particles in the respirable range are the most important from the toxicological point of view, the size distribution should also be determined and taken into consideration. The size distribution is determined with microscopy, liquid sedimentation or, during the sampling, with cyclones.

Interpretation. The most important sampling problem is to ensure the representativeness of the sample. Results obtained during one day, or from a few sites only, cannot be given too much weight. Monitoring must be regular if reliability is to be ensured, and plotting the results of successive measurements on a control chart is recommended. Needless to say, the analytical methods used should be strictly standardized and quality control programs should be employed both within the laboratory and between laboratories.

Only a broad outline of monitoring has been presented here. For more detailed data the reader is referred to specific textbooks or monographs on the topic (e.g. Patty, 1962; Fairhall, 1969; Rossano, 1969; Olishifski and McElroy, 1971; Roach, 1973; Linch, 1974) as well as to the chapter on analytical methods and sampling in the present volume.

4. General environment

Many metals are essential to life in small amounts, but toxic in higher doses. Others, such as lead, mercury, and cadmium, probably lack physiological function in man. Since all metals occur naturally in the earth's crust, an environment completely without exposure has never existed. However, civilization, especially during this century, has caused a sharp increase in the concentration of many metals in the air, soil and water (e.g., Patterson, 1970). The main sources are industrial emissions, traffic, burning of fossil fuels, and the weathering of metallic constructions and paint. Moreover, man-made products containing toxic metals as such, or in the form of alloys and compounds, are coming into the market in ever increasing numbers. Because of the wide use of such products, the likelihood that the general public will be exposed to toxic metals is strong.

Exposure levels vary greatly, depending on geographical, cultural and other circumstances (e.g. WHO, 1973). For example, urban environments are more polluted than rural ones, and industrialization increases exposure directly through emissions and indirectly through its products. Local habits, for instance, the use of lead-glazed ceramic containers, may further cause exceptionally high exposure (NAS, 1972). Finally, enrichment may take place in biological chains, such as in the case of mercury (Friberg and Vostal, 1972).

Preventive measures as far as environmental exposure is concerned have two goals, namely the prevention of poisoning in the presently living population and the reduction of metal accumulation in the environment so that future generations are also protected. The risk for poisoning in the presently living population is obviously highest in connection with massive exposure from air, food or drinking water. The Minamata and Niigata episodes are perhaps the best known, but even worse mass poisonings have occurred. In 1972 there was a severe outbreak of methylmercury poisoning in Iraq. According to official sources, 459 patients died, the total number of hospitalized patients being 6530 (Bakir et al., 1973). In the United States it is estimated that several thousand children each year contract lead poisoning from ingesting flaking paint (Moore, 1970; Guinee, 1971). Old water pipes made of lead and tanks painted with lead oxide may mean a risk for poisoning from drinking water. This has been reported in a district of Glasgow (Beattie, 1972), and probably occurs elsewhere as well.

There is so far no evidence that leaded gasoline causes poisoning in the general population (NAS, 1972). However, because the practice results in continuously increasing dispersion and accumulation in the entire environment, authorities in several countries have made moves to reduce or ban the use of organic lead as an additive in order to protect future generations.

Generally speaking, it is extremely difficult or even impossible by man-made means to eliminate metals that have already become spread. On the other hand there are many ways to treat current pollution problems. Because of the manifoldness of both exposure and the methods for reducing exposure outlets, only broad guidelines for preventing metal poisoning from the general environment can be given.

Metals already spread. In some very exceptional cases it is possible to remove metals already dispersed in the environment. There are large campaigns in the United States, in some states backed by law, aimed at removing lead paint from dwellings, either through the reconstruction of whole areas, or through urban renewal (NAS, 1972). Of course, the lead is not completely removed from the environment, since it is difficult to avoid transportation to rivers and lakes through sewage systems. However, the most dangerous hazard, namely, that of children ingesting flaking paint, can be eliminated. Old lead plumbing systems can be reconstructed with safer materials. Though, aside from a few cases, it is generally not possible for man to influence significantly the distribution pattern of metals already dispersed, natural processes may do so. Rainfall removes metals from the air, and sedimentation redistributes metals in water systems (NAS, 1972; Friberg and Vostal, 1972; Friberg et al., 1974).

Source control. The most effective method of control is of course the discontinuation of dangerous and dispensable use. Such a step also reduces the need for production and hence eliminates the exposure of the worker. Generally, such a drastic measure requires a legislative ban, as in the case of the use of methylmercury compounds for seed dressing in Sweden in 1966 (Abramson, 1967). Sometimes a practice is abandoned voluntarily, as in Finland in 1969 when the pulp industry agreed to discontinue the use of phenylmercury acetate for slime control. Another measure would be to recover the metals for reuse as soon as they escaped from industrial processes. This is seldom economically attractive, however.

In general, then, control is based on the prevention of escape at the source of pollution. In principle, there are two types of escape, namely, "economical," i.e., metals are emitted during refining, smelting or industrial use, and "noneconomical " i.e., impurities are released during a process. "Economical" escape into the air occurs from smoke stacks, exhaust ventilation systems and, to a lesser extent, from whirled-up dust. Hence the most important method of preventing such emissions is the purification of smoke and exhaust. The methods vary immensely, but the following four principles are fundamental to most methods of particulate control (Elvingson, 1972):

- separation by gravity forces (e.g., cyclones),
- separation by dissolution (e.g., water scrubbers),
- separation by filters, and
- separation by electrostatic powers (electrofilters).

In addition cooling traps and absorption into organic polysulfides are used for capturing mercury vapor.

The aquatic system becomes contaminated when industries discharge waste directly into it (especially small factories often dispose their sewage water into community sewers without any purification), when rainwater washes away the metal-containing dust that has settled around industries, and when metal trapped in air purification systems is carelessly handled. Water scrubbers are a particular problem in the last respect. The purification of sewage water is based on recirculation and various precipitation processes. For example, mercury can be precipitated into sulfide and absorbed into aluminum hydroxide, which is then removed as a foam. Iron oxide can also be used. Unfortunately, the control of the spread of dust from the surroundings of industries sometimes entails a liberal use of water, thereby placing an extra load on water cleaning systems and in some cases, polluting rivers and lakes.

The most important "noneconomical" escape of metals into the air takes place during the burning of fossil fuels, e.g. contributing twice as much mercury to the atmosphere as do emissions from industry (MacGregor, 1975). Large conventional power plants are the main polluters. The largest power station in Czechoslovakia uses coal containing 1,530 mg of arsenic compounds per kilogram. About 138 tons of arsenic enter the atmosphere each year from this single source (Cmarko and Rosival, 1970). Home heating also causes metal emissions.

Small amounts of some toxic metal occurring together with a less toxic one may undergo a "noneconomical" escape into the environment. For example, cadmium is an impurity in materials containing zinc. Since the demands for controlling zinc emissions are more liberal than those for cadmium, excessive amounts of cadmium easily escape.

Even when the air is the first environmental medium to become polluted, both soil and water become polluted secondarily through dustfall or washout by rain. Around the Czech power plant mentioned above, high concentrations of arsenic (up to 0.21 mg/l) have been measured in surface water (Cmarko and Rosival, 1970).

Pollution is usually the greatest around industries and other major polluters. Hence the risk for adverse effects is the highest among those living in their vicinities. Taller chimney stacks would distribute the emissions over larger areas and thereby dilute the concentrations, but this practice is no substitute for efficient exhaust purification in the case of toxic metals and should therefore be condemned. The same can be said of the practice of leading unpurified sewage water containing e.g. mercury or cadmium far out into the sea.

While it is possible practically to control emissions from a large source of pollution, such as a power plant, purifying the smoke from single homes is not economically feasible. Massive changes such as the development of electrical heating or district heating systems may help to concentrate the pollution, thus rendering purification procedures more feasible.

Waste and scrap. Pollution of the soil and water systems may arise from the weathering of disposed metal products. The reuse of scrap decreases both such pollution and the need for exploiting ores. However, most scrap for reuse goes to iron and steel works where the melting process releases impurities (e.g., cadmium and lead) stemming from alloys, paints, etc. The impurities are seldom regained but are emitted into the environment with the smoke. Unless effective exhaust purification is applied, such recycling in fact increases rather than decreases exposure to toxic metals.

Metal objects such as cans and capsules often end up in community incinerators together with other waste. Burning such waste may cause significant emissions of the most common toxic metals, especially lead.

Careless dumping of metal waste can severely pollute water basins. For example, old gravel quarries are definitely unsuitable as dumps since dissolved metal compounds have easy access to the ground water. All dumps must be without any contact with water supplies. A specific problem has arisen as sewage sludge has become used as fertilizer. Such sludge may contain fairly high concentrations of many metals, e.g. cadmium, which may then enter the food chain (Friberg et al., 1974).

Contaminated food and beverages. The soil, water and vegetation near large smelters or other major emitters are often heavily contaminated with metals (WHO, 1973). Vegetables and grain grown in such environments usually contain undesirably high concentrations of metal, just as the vegetation near busy highways has shown high lead levels (NAS, 1972). Community and well water often becomes contaminated (WHO, 1973). Preventing undue exposure from such sources requires, in addition to reducing the emissions, the monitoring of food and water, and a ban of the sale of foodstuffs containing excessive amounts of metals. Food and wine may also be contaminated from lead-glazed ceramic ware, which releases large amounts of lead in an acid milieu. In addition, canned food may include lead that has been released from the containers. Several cases of cadmium poisoning have occurred due to the ingestion of food and drink into which this metal has dissolved from cadmium-plated articles, e.g., ice trays, juice makers, roasting pans, etc. The problem of fish contaminated by methylmercury has already been mentioned. Other toxic metals, e.g. cadmium and arsenic, have also been found in high amounts in seafood in some areas. Preventing poisoning from such foods requires systematically organized analyses of their metal contents. Ceramic ware should be tested for lead release during acid treatment before being allowed on the market.

The consumption of illicit whiskey, often distilled with discarded car radiators or lead-soldered tubings, causes many cases of severe poisoning each year in the United States (Hernberg, 1975) and perhaps elsewhere. Effective consumer information is probably the only method for preventing such poisoning, since the trade seems to be flourishing in spite of legal efforts to eliminate it.

The effect of lead plumbing on tap water has already been mentioned. It should be added that artificial softening of water will increase its lead content. According to WHO (1973), most tap water does not contain excessive amounts of metals, with the possible exception of cadmium.

Accidents. There are almost unlimited possibilities for the accidental intake of toxic metals. For example, children who have sucked lead toys or swallowed small lead items, such as curtain weights, have even been fatally poisoned (Bacon, 1967; Fristedt, 1968). The preventive action lies in banning the use of toxic metals both as components and as paints, in such consumer goods as toys. When thermometers are broken indoors, especially in bedrooms, hazardous mercury concentrations may arise unless the spilled droplets are meticulously removed, e.g., with the aid of sulfur powder. As already mentioned, what can happen when mercury-treated grain is consumed by mistake has been experienced in Iraq.

Environmental monitoring. The need for monitoring metal concentrations in food and drinking water, whenever there is reason to suspect excessive amounts, has been stressed. In addition, air and water monitoring help ensure that regulations are being met. In principle, two types of sampling procedures should be used. First, metal concentrations should be measured at representative sites so that environmental standards can be controlled. Second, emissions from particular pollution sources should be checked so that individual polluters will comply with emission standards.

5. Medical aspects of prevention

Though the practice and the ambition level differ, the principles of medical prevention of effects of toxic metals are the same in public and occupational health, namely,

- identification of risk groups,
- biological monitoring,
- health examinations,
- early removal of the subject from dangerous exposure, and
- early treatment, if possible.

Identification of risk groups. Advance knowledge of where a risk is apt to occur is of paramount significance to the identification of risk groups. Some of these groups have already been pointed out, i.e., specific groups of industrial workers, populations living around smelters and other emitters (true for all toxic metals), slum district children (lead), consumers of illicit whiskey (lead), heavy fish consumers (mercury,) dwellers in districts with lead plumbing systems (lead), and users of primitive ceramic ware (lead). In these population groups health screening procedures can be recommended; however, such procedures are not warranted or possible among the population at large.

Biological monitoring. In industry biological monitoring has been carried out for decades, but in public health no regular programs exist. Hence most experience from the latter sphere is derived from ad hoc campaigns, such as the case finding projects in the USA (lead) and the studies of fish consumers in Sweden and Japan (mercury).

The choice of indicators and biological samples varies from one metal to another. In general, the parameter of exposure, i.e., the concentration of the metal itself in some biological medium, is the only one available because special tests measuring response are lacking. Lead is an exception. Because of its specific effects on blood formation, several good parameters of response can be utilized. There are several metals, such as arsenic, chromium, nickel and manganese, for which sufficient information regarding the significance of some given level in biological media is also lacking. Consequently, while determinations of these metals in biological media are technically possible, the results are difficult to interpret. Hence, recommendations for the routine biological monitoring for these metals cannot yet be given.

At present lead is more suitable for biological monitoring than other metals since the dose-effect and dose-response relationships are fairly well known. The concentration of lead in blood describes current exposure better than any other test. Measurement of lead in blood should be preferred to that of e.g. lead in urine. Exposure to organic lead is an exception; lead in urine is the measurement of choice in this case (Hernberg, 1975). When a low enough action level is selected (e.g. 50 or 60 μg/100 ml), enough safety can be ensured if blood lead alone is used for health monitoring. If the chosen action level is exceeded, the worker should be removed from exposure and submitted to additional tests measuring response. The most widely used ones concern the concentrations of delta-aminolevulinic acid and coproporphyrin in urine. Quite recently the determination of erythrocyte zinc protoporphyrin has become available as a routine method (Fischbein et al., 1976). This may become the method of choice in the future, even for indirectly monitoring exposure, because of its simplicity and good reflection of "active" lead. Its major weakness is the time lag of some weeks that occurs before the concentration rises due to the relatively slow turnover of erythrocytes. Dangerous, new exposure may thus pass undetected if monitoring relies only on this test. For screening the general population, such as persons living around lead smelters, the determination of erythrocyte delta-aminolevulinic acid dehydratase activity is more suitable because of its high sensitivity. It is also cheaper and has higher analytical accuracy than blood lead determination (Berlin et al., 1973). Because of the poor stability of the enzyme activity, the analysis must be performed within a few hours.

In the case of mercury, exposure tests are less reliable. Mercury in urine has been the most widely used exposure test in industry when inorganic mercury has been the main type of exposure. Recently, mercury in the blood has also come into some usage. Methylmercury is excreted very slowly in the urine, for which reason this medium is not suitable for monitoring exposure to this compound. Instead, blood and hair samples are good indicators (see Berlin, 1977).

Cadmium can be measured in both blood and urine but the physiological significance of some given concentrations is not yet well known. It is believed that cadmium in blood reflects current exposure while cadmium in urine reflects body burden. Hence both blood and urine tests should be used concurrently (Task Group on Metal Toxicity, 1976).

Regulations requiring biological monitoring concern occupational exposure only. Many countries have such regulations for lead. In principle, the frequency of monitoring is determined by the degree of risk. For example, according to Finnish regulations, blood lead determinations should be made from one to six times a year, the frequency depending on the type of industry. Workplaces such as printing shops or garages need only one monitoring annually while high-risk occupations such as lead smelting and battery manufacturing require six. These are minimums; the physician responsible has the right to require more frequent monitoring, if he considers it necessary (Tola et al., 1976).

Health examinations. Health examinations are clearly warranted in occupational health and can be recommended as a tool for preventing lead poisoning in children living in slums. Obviously, they are meaningful only when exposure is regular; accidental poisoning can never be prevented by health examination programs. In occupational health there are two types of examinations. Preemployment examinations aim at excluding from exposure workers who for some reason would be especially sensitive to toxic metals. Periodical checks are programmed to detect toxic effects of metals, at an early stage, as well as to discover other diseases, since the latter may weaken the worker's resistance. Special attention should be paid to the critical organs involved, i.e., the hematopoietic and nervous systems in lead exposure, the nervous system and the kidneys in mercury exposure, and the kidneys in cadmium exposure. In addition the information gained from biological and environmental monitoring should be utilized.

The frequency of periodical examinations may vary depending on the degree of risk - usually one examination a year is enough - provided prompt action is taken whenever the action level is exceeded.

Cessation of exposure. The most important early "therapy" for metal poisoning is the cessation of exposure. Usually no further treatment is needed if exposure is interrupted early enough. Although this is scientifically hard to defend, a dualism generally exists between the action levels in occupational and public health, the action levels forthe former being more lenient. Removal of the worker from exposure should be considered when the concentrations of the metal in the blood or urine exceed the action level, when early symptoms or signs are present or when reports of exceptional exposure peaks are made. Ideally, the worker should be transferred to a job that is free from exposure. Otherwise, sick leave must be considered. The worker should not be allowed to return to exposure until the blood or urine levels of the metal are well below recommended upper limits. In the general population, which is not under regular supervision, the policy is to interrupt potentially hazardous exposure under less urgent circumstances than the ones mentioned above.

Because of the potentially severe outcome, cessation of exposure is even more important in such cases as when children have ingested flaking lead paint. It is best to hospitalize the child while the home is being sanitized. If possible, the emotional disturbances underlying the pica syndrome should also be treated (Chisolm and Kaplan, 1968).

Drugs, vitamins and special diets. The prevention of metal poisoning calls primarily for technical solutions but under special circumstances, certain adjuvants to technical prevention may be considered. In developing countries where the nutritional state of workers is poor, it is important to ensure a diet adequate in vitamins, minerals and protein. It is urgent that the persistent myth that milk protects against lead poisoning be abandoned, as there is no rationale for it. The tendency for pica that exists among slum district children is often due to iron deficiency; correcting this nutritional deficiency may reduce the risk of lead poisoning.

Interactions and competition between metals alter their toxicity. For example, zinc decreases many of the toxic effects of cadmium, and also of lead (Border et al., 1976; Abdulla and Haeger-Aronsen, 1973) and calcium deficiency inreases the absorption of lead from the gut (Task Group on Metal Accumulation, 1973). While dietary and interactional factors must be kept in mind and improvements made in developing countries whenever minimum daily requirements are not reached, attention should not be diverted from the only correct preventive strategy, namely, that of improving industrial hygiene.

Finally, the prophylactic use of chelating agents deserves some comments. This malpractice is astonishingly widespread in several European countries and in the United States (Lilis and Fischbein, 1976). There are several reasons to condemn this form of "prophylaxis". First, it provides the employer with too easy a way of continuing to have poor workplace hygiene. Second, all chelating agents also mobilize essential metals, such as zinc and copper (Lilis and Fischbein, 1976). Third, all such agents have adverse side effects, such as nephrotoxicity (pencillamine and EDTA), myelotoxicity (penicillamine), and they may be allergenic (penicillamine) (Lilis and Fischbein, 1976). Fourth, oral administration increases absorption from the bowels. For these reasons no serious physician should consider the prophylactic use of chelating agents. The treatment of metal poisoning is quite another matter.

ACKNOWLEDGMENTS
The author wishes to thank Mr. Antti Tossavainen, L.Sc. (Tech.), Mrs Pirkko Pfäffli, M.Sc., and Dr. Sakari Tola, M.D., for their constructive criticism of the manuscript.

REFERENCES

Abdulla, M. and Haeger-Aronsen, B. (1973). International Research Communication System - General Pharmacology 73-8, 8-14-1.

Abramson, E. (1967). Läkartidningen 64, 3638-3640.

ACGIH (1974). "Threshold Limit Values for Substances in Workroom Air." American Conference of Governmental Industrial Hygienists, Cincinnati.

American National Standard for Respiratory Protection Z88.2-1969 (1969). U.S. Standards Institute, New York.

Bacon, A.P.C. (1967). Lancet 1, 264-266.

Bakir, F., Damluji, S.F., Amin-Zaki, L., Murtadha, M., Khalidi, A., Al-Rawi, N.Y., Tikriti, S., Dhahir, H.I., Clarkson, T.W., Smith, J.C. and Doherty, R.A. (1973). Science 181, 230-241.

Beattie, A.D., Moore, M.R., Devenay, W.T., Miller, A.R. and Goldberg, A. (1972). Brit. Med. J. 2, 491-492.

Berlin, M. (1977). In: "Toxicology of Metals." Volume II, pp 301-344. Environmental Health Research Series, 600/1-77-022, Environmental Protection Agency, Research Triangle Park.

Berlin, A., Del Castilho, P. and Smeets, J. (1973). In: "Environmental Health Aspects of Lead." pp 1033-1049. Commission of the European Communities Directorate General for Dissemination of Knowledge, Center for Information and Documentation CID, Luxembourg.

Border, E.A., Cantrell, A.C. and Kilroe-Smith, T.A. (1976). Brit. J. Ind. Med. 33, 85-87.

Chatterjee, B.B., Williams, M.K., Walford, J. and King, E. (1969). Amer. Ind. Hyg. Assoc. J. 30, 643.

Chisolm, J.J., Jr. and Kaplan, E. (1968). J. Pediatr. 73, 942-950.

Cmarko, V. and Rosival, L. (1970). Preprint to Conference on Chemical Pollution and Human Ecology, Prague, 1970.

Elvingson, C.O. (1972). In: "Environmental Science in Practice." (P. Brink, T. Céwe, A. Horn af Ranzien, E. Olerud, C. Ramel and H. Sjörs, eds) pp 172-205. Centraltryckeriet, Borås, Sweden (In Swedish).

Fairhall, L.T. (1969). "Industrial Toxicology." 2nd edition. Hafner Publishing Company, New York.

Fischbein, A., Eisinger, J. and Blumberg, W.E. (1976). Mount Sinai J. Med. 43, 294-299.

Friberg, L., Piscator, M., Nordberg, G.F. and Kjellström, T. (1974). "Cadmium in the Environment." CRC Press, Cleveland.

Friberg, L. and Vostal, J. (1972). "Mercury in the Environment." CRC Press, Cleveland.

Fristedt, B. (1968). Läkartidningen 65, 3028-3034.

Guinee, V.F. (1971). Trans. N.Y. Acad. Sci. 33, 539.

Hamilton, A. and Hardy, H.H. (1974). "Industrial Toxicology." 3rd edition. pp 134. Publishing Sciences Group, Acton, Mass.

Hernberg, S. (1973). Work Environ. Health 10, 53-61.

Hernberg, S. (1975). In: "Occupational Medicine: Principles and Practical Applications." (C. Zenz, ed) pp 715-770. Year Book Medical Publishers, Chicago.

Japanese Ministry of Health and Welfare (1967). Report on the cases of mercury poisoning in Niigata, Tokyo (mimeographed document).

Jensen, S. and Jernelöv, A. (1969). Nature 223, 753-754.

Lilis, R. and Fischbein, A. (1976). J. Amer. Med. Assoc. 235, 2823-2824.

Linch, A.L. (1974). "Evaluation of Ambient Air Quality by Personnel Monitoring." pp 121-170. CRC Press, Cleveland.

MacGregor, A. (1975). Environ. Health Perspect. 12, 137-148.

Moore, J.E. (1970). Amer. J. Public Health 60, 1430.

NAS (1972). "Airborne Lead in Perspective." Committee on Medical and Biological Effects of Environmental Pollution, National Academy of Sciences, National Research Council, Washington, D.C.

NIOSH (1972). "Criteria for a Recommended Standard ... Occupational Exposure to Inorganic Lead." U.S. Department of Health, Education, and Welfare, Public Health Service, Health Services and Mental Health Administration, National Institute for Occupational Safety and Health.

Nordberg, G.F., ed (1976). "Effects and Dose-Response Relationships of Toxic Metals." Elsevier, Amsterdam.

Olishifski, J.P.E. and McElroy, F.E., eds (1971). "Fundamentals of Industrial Hygiene." pp 439-456. National Safety Council, Chicago.

Patterson, C.C. (1970). Proc. Amer. Phil. Soc. 114, 9-12.

Patty, E.A. (1962). "Industrial Hygiene and Toxicology." 2nd revised edition, Vol. I: "General Principles." Interscience Publishers, New York.

Roach, S.A. (1973). In: "The Industrial Environment - its Evaluation and Control." pp 139-155. U.S. Department of Health, Education, and Welfare, NIOSH.

Rossano, A.T., ed (1969). "Air Pollution Control Guidebook for Management." Environmental Sciences Service Division, E.R.A., Stamford, Conn.

Task Group on Metal Accumulation (1973). Environ. Physiol. Biochem. 3, 65-107.

Task Group on Metal Toxicity (1976). In: "Effects and Dose-Response Relationships of Toxic Metals." (G.F. Nordberg, ed) pp 1-111. Elsevier, Amsterdam.

Tola, S., Hernberg, S. and Vesanto, R. (1976). Scand. J. Work Environ. Health 2, 115-127.

WHO (1973). Long-term programme in environmental pollution control in Europe. The hazards to health of persistent substances in water. Annex to a report of a working group. Technical documents on Arsenic, Cadmium, Lead, Manganese and Mercury, Helsinki, 10-14 April, 1972. WHO Regional Office for Europe, Copenhagen.

Williams, M.K. (1975). J. Occup. Med. 17, 93.

Williams, M.K., Walford, J. and King, E. (1968). Brit. J. Ind. Med. 25, 144.

Williams, M.K., King, E. and Walford, J. (1969). Brit. J. Ind. Med. 26, 202.

Ziegfeld, R. (1964). Arch. Environ. Health 8, 14.

GENERAL ASPECTS AND SPECIFIC DATA ON ECOLOGICAL EFFECTS OF METALS

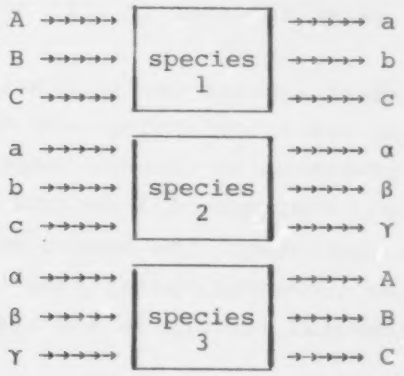
Karin Beijer and Arne Jernelöv

1. General aspects of ecological effects

1.1 Equilibrium and balance in the ecosystem

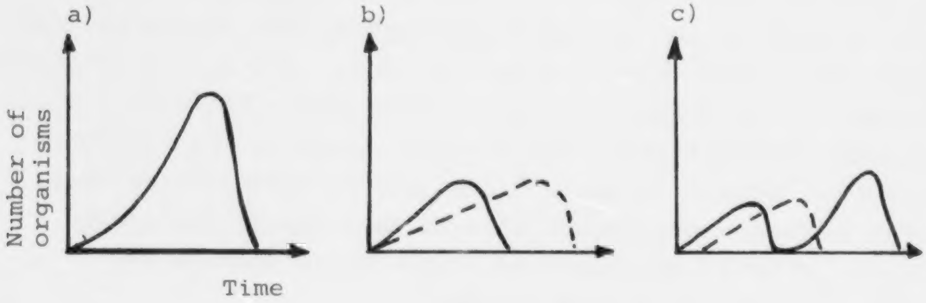
Ecology is the study of the reciprocal relations between living organisms and their environment. Each living organism affects its environment by consuming some substances such as water, solid foodstuffs and oxygen and by secreting others such as urine, feces, carbon dioxide etc. A specific organism, when confined on its own in a closed environment, tends to destroy the prerequisites for its own existence. The substances needed tend to diminish, while useless and sometimes poisonous waste heaps up in the surroundings. Different organisms have different metabolisms, however. That which is useless or harmful to one organism may be useful to another. One of the most obvious examples is that plants use carbon dioxide and water and give off oxygen while animals use oxygen and give off carbon dioxide.

In order to subsist, each ecosystem must recirculate all substances that are not regularly supplied from outside. This leads to a balance between the organisms which are part of the system and the relevant physical-chemical parameters. The schematic figure below illustrates the above mentioned rule.



Preceding page blank

Since each species (or individual organism) shows a tendency to destroy the prerequisites for its own existence it is evident that the species (or individual) cannot live alone in a secluded system. An experiment with the growth of microorganisms in a closed cultivating duct with a given substrate or a given quantity of oxygen illustrates this. At first the growth of the population is exponential, whereafter it slows down; finally, the population breaks down and dies out, see Figure a. If an additional species is introduced into the system and this species is a predator of the first, the growth curve in Figure b or the one in Figure c will be obtained.



If the number of species in a food-chain increases or the number of the environmental parameters regulating the number of individuals increases, the probability for a balanced system will increase as well, i.e. the number of individuals of a species will never be so low that it will be eliminated.

Obviously the different species can interact in many ways, for example by using and transforming each other's excretion products, through predation or through competition for scarce factors. Also, environmental factors interact with organisms and with each other. The number of simple interaction possibilities increases faster than the number of both species and environmental factors in the system. The interactions will equal n(n-1)/2 where n is the number of species and environmental factors. The interactions can be said to stabilize the system - e.g. by tending towards an even distribution of individuals among the species.

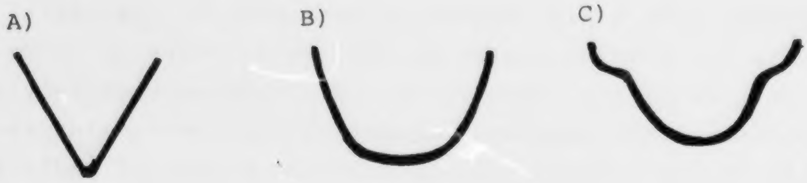
An ecosystem with a high degree of constancy in physical parameters and a large number of species is referred to as having high stability. Conversely, an ecosystem with varying (and unpredictable) physical parameters and few species is characterized by a large variation in the number of individuals within the species and is referred to as having low stability. This does not necessarily imply that the stable high diversity ecosystem is more tolerant to additional stress than one of low diversity already subject to stress.

The relationship between species and the number of individual organisms on the one hand, and the degree of diversity and instability on the other, have been used to characterize an ecological system. Many mathematical formulae have been used to express this relationship in the form of diversity indices, e.g. the Shannon-Wiever function, Simpson's index, Sander's rarefaction technique (Pielou, 1969; Fager, 1972).

Reduction of species diversity (changes in indices) when passing from moderately to strongly polluted parts of an area is frequently used in pollution ecology to measure the stress to which the ecosystem is being subjected.

Because of the interdependence of all included species and environmental factors a change in any parameter will have consequences for all the other parameters. The consequential changes as a rule tend to neutralize the effects of the primary change so that the system may return gradually to its earlier point of balance. Sometimes, the system will instead come to an altogether new point of balance.

The following lines illustrate different types of ecosystems with different capacities for "balance" and "stability":



The lowest points of the curves are the balance points. The horizontal curve sections with slight slopes constitute the amplitude of oscillation, and the vertical difference in altitude. The ecosystem may be regarded as a ball which is removed from the balance point and which later on falls back to the same point.

System A is obviously very stable. It has a given point of balance to which the system is rapidly brought back if disturbed. Much energy is required to disturb such a system.

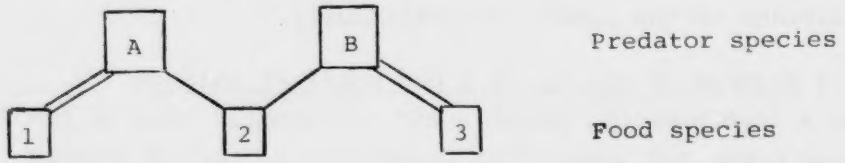
System B has a less marked point of balance and can be disturbed quite easily. The system will thus oscillate around the point of balance before being stabilized.

System C, finally, has no specific point of balance - it has a "balance area" within which no intrinsic force will move or maintain the system. When moved outside the "balance area" it will be brought back to it.

It should be pointed out, already at this stage, that knowledge about a system's point of balance, amplitude and stability - e.g. the shape of the curve within a certain interval - does not tell us about its reaction to strong interferences. That is, it is possible to interpolate earlier observations but never to extrapolate them. Moreover, the distinction between one system and another where a new species or environmental factor has disappeared or been added is often subtle or vague, but according to the above definition the new system has other properties. Many of the interactions between organisms are of a regulating feed-back type, which is one of the reasons for their stabilizing effect on the system.

1.1.1 Examples of stabilizing interactions

An ecological system consists of five species of which two are predators. Each predator lives on two of the other three species.



The number of predators is regulated by the food supply and the predators usually show a preference for eating the most commonly available food species. The total number of the food species individuals is regulated by the supply of nutriment and the relative number of the relative predation pressure. Now, let us assume that all three food species and the two predators are equally numerous to start with, and as a result of an external disturbance imposed on the system, the number of individuals of food species 1 is strongly reduced. Predator species A will then turn to eat principally 2. At the same time, the decreased total food supply causes the number of A to diminish relative to B. Owing to the fact that both A and B (A principally and B partly) now eat 2, the number of 2 is reduced. B then starts to eat mainly 3 while A continues to eat 2 since it is still the most common of the food factors. When A eats 2 and B eats 3, the number of 1 can increase again while the number of 2 and 3 decreases. There are now more B than A, because of the earlier adaptation to the food supply, and the predation pressure is found to be strongest on 3 which accordingly diminishes most rapidly. When 1 becomes more numerous than 2, A will proceed to eat mainly this species, and when 3 becomes rarer than 2, B will proceed to eat 2. Now that the food supply for A is more abundant than that for B, A will increase in number while B decreases.

In this way the number of the different organisms oscillates with a consequential successive reduction of amplitude until the system gradually recovers its earlier equilibrium. The interaction between the organisms contributes to the stability of the system. Thus, the mutual influence of the organisms in this kind of system tends to reduce the amplitude of the variations in the number of individuals.

1.1.2 Example of spiral to a new point of balance

Take a fish species, which lives the greater part of its life in the ocean but spawns and subsequently dies in running fresh water - in the same river from which it migrated after its first year. Provided the time period the fish needs to reach sexual maturity is relatively constant and spawning occurs only once, the fish will gradually tend to split up into subpopulations separated by different spawning years.

We assume that the number of individuals in a population is primarily limited by the number of fish surviving the first year in the river. For the sake of simplicity we further assume that the limiting factors are predators and supply of food organisms. In every natural population some variations in the number of individuals will occur.

A numerous offspring of fish in the river one year tends to cause the food organisms to decrease and the number of predators to increase because of the abundance of prey. As a consequence, the following year, the fry smolt will encounter a scarcity of food and a larger number of predators and thus fewer fish will survive in the river that year. The year after that the food will be abundant and the predators few because of starvation or migration which means a good year for the fish and so on. Thus the annual subpopulations will have different sizes.

This type of increased cyclic variation need not express unstability in the ecosystem. It merely emphasizes the complexity of the stability concept and shows that different development stages separated by some spatial dimension as well as regular migration should in some way be included in the concept "stability".

1.1.3 Ecological subsystems

Most ecological concepts are ambiguously defined. This applies to the word "system" as well. By way of introduction, the word "system" was applied in its theoretical meaning: a closed ecosystem in an area in which only the supply of energy in the form of light recirculates all substances and maintains all included types of organisms. Through the examples given above, the concept "system" has received a more practical-operational and thus a less strict meaning. All known ecosystems are more or less open and the majority consists of various subsystems. For the purpose of more exhaustive studies a lake as an ecosystem is often divided into a number of subsystems as for instance: two different littoral zones; the Phragmites and Nymphea zones, the lake bottom without vegetation, sometimes separated into soft and hard bottom, and the pelagial and profundale water bodies outside the littoral zones. Certain groups of animals, such as fish, migrate freely between these subsystems.

The subsystem can be internally unbalanced, but the development towards a breakdown in one of them will be checked at some given point by the surrounding subsystems - by external forces - and the balance thus restored.

In the discussion above it was assumed that the subsystems constitute different systems both physically and by appearance e.g. the littoral zone or the free water mass. The same argument may be held for a physically uniform system like e.g. the surface water - the pelagial.

In a fairly big lake or the oceans the pelagial constitutes, in one respect, a unit. Yet for an individual organism of the phyto- or zooplankton types, large parts of the space within this unit are beyond its reach. This would not be of significance if the organisms were distributed uniformly within the unit. However, this is not the case. Chance, if nothing else, will lead one to expect that some water subunits contain more plankton than others. Due to the limited mobility of these organisms one subunit, during a certain period of time, will constitute an ecological subsystem. During this period the migration in and out of the subunits will be of importance for the balance between the organisms. It is evident that the extension of such a closed unit both in time and space is rather vague.

Within these subunits with their different contents of organisms the development might proceed very differently. In some, the consumer organisms may eliminate the producers. In others, the consumers may be very scarce or totally absent and thus leave the producers to increase exponentially. This need not lead to a breakdown of the system as a whole since the durability in time of the subsystems is limited, and their borderlines shifting. This is an example of internal balancing.

In reality not only chance will cause an uneven distribution of organisms. The wind and currents, for instance, will affect groups of organisms differently according to their mobility, size, density and shape etc, leading to a much greater spatial separation than could be accounted for by chance only. This "patchiness" is a well-known phenomenon among plankton and also among benthic organisms.

From the above discussion it is clear that the concept of "balance" must be further extended and diversified. Balance in the total system does not presuppose a mathematical sum of balances in the subsystems. Spatial separation and the presence of non-discrete time and space dimensions make continued existence of the species possible.

1.2 Disturbance of ecosystem stability

The concept of biological effects of a pollutant includes changes in the individual, the whole population and the ecosystem. Most of the research in this field has so far been directed towards the individual. This is the obvious approach when studying effects in humans but it is impossible to predict the effects on an ecosystem from knowledge about the effects on individuals from an isolated species.

The effect produced in the individual by a harmful substance, may or may not affect the whole population (cf. the discussion on "litter mate-correlation" below). If the whole population is affected this in turn may or may not -depending on in what way the population is hit - affect the concentration in the organism of the substance in question. Thus one possible effect is a negative feed-back where the final result may be a steady state as for instance in the hypothetical example: high concentrations in fish → reduced reproduction → less competition for food → a more rapid growth → lower concentrations in the fish → improved reproduction ....

Another conceivable effect is a kind of vicious circle or spiral with the total elimination as the final result: high concentration in the fish → impaired growth → higher concentrations → further impairment of the growth → even higher concentrations ....

A number of examples can be given which show that the lack of ecological understanding leaves us without a guide as to the consequences of a pollutant.

We might for instance want to find out the effects of a temperature increase in a lake on a certain fish species. In laboratory tests it is found that, given enough food, the fish will grow faster and give more offspring when the temperature is raised. Thus it can be predicted that the result would be more and larger fish in the lake. But in reality this might not be the case at all. If there are other species even more favored by the changed conditions, these will take over through competition for food and as a consequence our species might even become totally eliminated.

An attempt to anwer the question about the effects on a fish population if 25% of the brood is eliminated will give rise to a new question as to whether the "litter mate-correlation" - that is the probability for survival of one individual when affected by the death of another - is positive or negative.

For decades attempts have been made to increase the population of pike in Swedish lakes through the introduction of fry. These have shown that the survival of the individual mainly is dependent on the competition for food with other fry of the same age. Thus if the number of fry is increased the probability for survival of the individual decreases. It therefore seems likely that the probability for survival of the individual would increase with a decreasing total number. The conclusion would be that the effect on the adult population of this kind of fish species through a 25% kill of the fry would not be drastic.

However, in the case of grouper species like e.g. herring the effects would most likely be severe on the adult population. The predators are always superior and take the number of fish they need. This may be an explanation for the development of shoals, since each individual fish will stand a better chance of survival when surrounded by many others of the same size.

Thus, if the number of fry is decreased due to increased competition for food, each fish will be in a statistically worse situation and the probability for it to fall prey to predation will increase. (Provided that food is abundant enough so that the forming of shoals will not have a negative effect on the growth. Where there is a scarcity of food a decrease in the number of fry would leave more food per individual and thus promote a higher growth rate. This would give rise to yet another unpredictable situation.)

From these two examples it can be seen that knowledge on the litter mate-correlation is necessary for drawing conclusions when testing toxic effects on fish fry or disturbances on reproduction. However, this relationship is very rarely known or possible to guess.

If the balance between zoo- and phytoplankton is based on a feed-back system where a decrease of phytoplankton - food - leads to fewer eggs for the zooplankton which means less grazing, this in turn leads to an increase of phytoplankton which means more eggs etc. The system is very sensitive to disturbances of reproduction. If, on the other hand, the total balance is kept up through the interaction of unbalanced subsystems separated in space, the effects would not be so serious.

Usually it is not known through what principles the ecological balance is maintained. Thus the impact on the ecosystem as a whole of decreased reproduction of a certain species caused by a pollutant cannot be predicted. Like in the examples above, different hypotheses concerning the premises for an ecological balance lead to diametrically opposed conclusions about the ecological consequences of a certain effect on the individual.

1.3 Accumulation of trace substances in biological systems

The concentration of a substance in a biological system - be it an organ, an individual, a population, a trophic level etc. - is the result of input, dilution and output. Bioaccumulation of a substance will occur when the rate of uptake exceeds that of transformation, dilution and/or elimination. Any substance with such physico-chemical properties that it may be transported along with the flow of material may potentially be accumulated in a biological system. Biomagnification is the increase of the concentration of a trace substance on a body weight basis in successively higher trophic levels - e.g. plankton → fish → seal. A prerequisite for this is usually that a major uptake route is via the food and that the substance is accumulated. Thus, in the aquatic environment, where the uptake of many trace substances from the ambient water is of importance, the lifespan and growth rate of an organism may be of greater significance for the concentration than its position in the food chain.

Fagerström and Åsell (1973) have discussed a general methodology for modelling bioaccumulation phenomena. It is suggested that any process in a biological system that will result in a transport of a trace substance should be considered in terms of a carrier-trace substance complex. The following are some points made in their paper:

Through a biological system there is a flow (continuous or intermittent) of a carrier. This may be a well defined chemical species, such as calcium or oxygen or a chemically less specific concept, such as "lipids" or "energy". It is essential that the carrier flow is a process which is a normal and necessary part of the metabolism of the system, having its own regulatory mechanisms in order to maintain a sufficient degree of homeostasis.

Through the system there is also a flow of a trace substance with such chemical properties that it acts more or less as an analogue to the carrier. This means that it traces the carrier flow in the sense that it is being passively transported and stored by the carrier. The trace substance may be a radionuclide such as ^{90}Sr, a pesticide such as DDT, a metal such as mercury etc.

The rate of flow of the carrier through the system is affected by a variety of variables, endogenous as well as exogenous. For example, if the system considered is a fish specimen and the carrier is "energy", it is clear that the flow of energy is affected by such variables as temperature and body size.

Any flow of a carrier will cause a flow of the trace substance. If the trace substance were a perfect analogue to the carrier, the amount being transported per unit of carrier would always be the same as the amount present per unit of carrier in the source of intake to the system. For example the amount of methylmercury taken in by a fish per unit of food would be the same as the concentration of methylmercury in the food. This is not the case, however, since the analogy between food and methylmercury is far from perfect.

The summed effect of all flows into and out of a system, integrated over time, will determine the pool sizes of carrier and trace substance. Thus, it follows that if the trace substances were to act as an analogue to the carrier, the ultimate steady state situation in a food chain would be that the concentration of a trace substance relative to the carrier in the organisms would equal that in the source of contamination, that is, no accumulation in the food chain. This is the case with for instance ^{14}C where the isotopic effect is negligible and a constant input to the biosphere has been going on for a long time. In other cases where there is an analogy but where the contamination has started recently or is changing with time, the ecosystem may be in a transient state. In such systems the ultimate steady state situation is predictable when the ratio between the trace substance and the carrier in the source of intake is known. From this follows that the concentration calculated on a body weight basis of a trace substance - say ^{90}Sr - may be higher in one type of organism (e.g. a snail) than in another (e.g. plankton) although both have the same concentration with regard to the carrier system, in this case calcium.

Trace substances which will be accumulated (e.g. methylmercury) do not act as analogues to their carrier in most processes, such as transport over surface membranes and metabolism. This is basically the reason that they will be biomagnified in food chains. In other words: the fact that a trace substance may act as an analogue to a carrier in a biological system does not imply that it will be biomagnified in food chains. On the contrary, its very deviation from analogy, for instance by being more efficiently retained in a tissue than the carrier is, is what causes its accumulation. Therefore, the commonly used argument that a trace substance is magnified in food chains because of a ~ 90% loss of energy in each trophic level is misleading. It is not the 9:1 relation between metabolized and stored carrier as such that is important; it is the fact that the relation between metabolized and stored trace substance is different from (and less than) 9:1.

2. Effects of metals on ecosystems

Little is known about the gradual ecological changes which may result from metal contamination of the environment. It is generally true that effects will go unnoticed unless they are extreme. Also, there is very little knowledge about at what level in a certain organ a metal or a combination of metals start to be harmful to the individual and thereby to the ecosystem. In most cases, observed elevations in metal levels in the biota are our only indication of metal disturbances.

2.1 Terrestrial ecosystems

Metals reach the terrestrial environment through atmospheric fallout (wet or dry deposition), through irrigation with contaminated water, with pesticides and different types of fertilizers and also through the disposal of solid waste.

2.1.1 Soil

The influence of air-deposited metals on biological and biochemical processes in soil has been studied. It has been found that some metals, particularly copper, limit the decomposition rate of forest litter at concentrations of a few times the background level. Microbial decomposition of cellulose and starch, mineralization of nitrogen and phosphorus, and activities of a number of soil enzymes have also been shown to be disturbed by an increase of copper and zinc levels. The ultimate consequence of this may be a productivity decrease of the ecosystem (Tyler, 1975).

2.1.2 Vegetation

Metals are taken up by the plant roots and subsequently distributed to stems, leaves and seeds. The uptake of a metal is affected by soil organic matter content, pH, the presence of other metals etc. The extent of foliar absorption is difficult to determine but estimates indicate that it may not be very significant percentagewise for a number of metals e.g. Pb. Plant metabolism may be disturbed in many ways by toxic metals, resulting in reduced standing crops and fertility.

Some plants tolerate high soil levels of specific metals which they tend to take up and enrich, a fact that has been used by geologists for base metal prospecting. "Accumulator species" have been found for F, As, Si, V, Cr, Co, Ni, Cu, Zn, Se, Sr, Y, Sn, Ba, W, Re, Bi and U. Both Hg and Cd are highly toxic to most organisms and very few natural accumulator species have been found for these two elements (Peterson, 1971). However, experiments indicate that plants may readily absorb and translocate Cd to above-ground tissue when cadmium levels in soil are elevated (John, 1975). Cadmium can be enriched to a very high degree in crop species such as wheat and rice. Plutonium may be solubilized by soil microorganisms and taken up by plants (Drucker, 1976).

The enrichment of trace elements from sewage sludge fertilizer in soils and plants has been studied (Andersson and Nilsson, 1972). It was found that larger amounts of trace elements are added than removed when sewage sludge is repeatedly used on cultivated soils, leading to a gradual enrichment in the soil and for some elements an increased uptake by plants and transference to the food chains. The extractable soil content of Hg, Zn, Cu and Se was found to be increased more than 100 per cent and that of Ni, Cr and Pb more than 50 per cent. In vegetation the levels of Hg, Zn, Cu, Ni, Cr, Pb, As and Mg were increased by about 50 per cent or more. Similar experiments with corn plants have shown that Cr, Mn, Ni, Pb and Cu were not significantly affected by waste additions, whereas Fe, Zn and Cd were (Webber and Beauchamp, 1975).

The enrichment of toxic metals in the soils around smelters has been shown to result in denudation of forests and in the inability of plants to recolonize the exposed soils (e.g. Whitby, 1975; Hutchinson, 1975). In the environs of a zinc smelter also emitting Cd, Pb and As altered floristic composition and lowered species diversity could be observed. The degree of effects was found to be correlated with increased smelter proximity and with levels of the metals in both soil and vegetation (Benenati and Risser, 1975).

2.1.3 Fauna

Aerial emission of metals - especially from smelters - is known to have a damaging effect on livestock and other grazing animals. In connection with a study of atmospheric metal fallout, horses that probably died from metal poisoning were analyzed and found to have very high levels of some metals, notably lead and cadmium (Goodman and Roberts, 1971). As was shown by Schroeder and Balassa (1961) cadmium is accumulated in grazing animals, especially in kidney and liver. Westermark et al. (1973) found indications of a biomagnification of cadmium but not of arsenic in the terrestrial food chain.

Automobile exhausts are a major source of lead emission - both organic and inorganic. Kidney and liver levels in sheep grazing near a major road were found to be four to five times higher than in a control group (Lundström, 1971), and the level in milk produced by cattle fed on hay harvested along a road was found to be four times higher than normal (Bovay, 1971). The transport of lead in an arctic food chain was studied by Holtzman (1968). No biomagnification was demonstrated, probably because inorganic lead is stored mainly in bone and thus made less and less available to each successive trophic level.

It seems that the terrestrial fauna is to a large extent contaminated with mercurials through the ingestion of seeds treated with mercury-containing fungicides. Analysis of feathers from goshawk (Accipiter gentilis) shows a strong correlation between the introduction and then the withdrawal of alkyl mercurials on the one hand and the mercury levels in the feathers on the other. Levels were below 3 mg/kg before the introduction of mercurials, 20 mg/kg during their usage and 3 mg/kg after their withdrawal (Westermark and Sjöstrand, 1972).

In connection with the outbreak of mercury poisoning in Iraq in 1974, where people had been eating methylmercury-treated seed, wild life samples from different areas were analyzed for mercury content (Jernelöv, 1974). Tail feathers from seed-eating birds were found to contain 13.5-21 mg/kg of mercury which is about ten times higher than values reported from Ethiopia and within the range found in Sweden and Canada. Extremely high concentrations of mercury were found in muscle tissue (up to 40 mg/kg) and feathers (up to 52 mg/kg) of dead seed-eating birds. These very high levels were only found in the vicinity of storage houses where returned seed was kept. No predatory birds could be caught and analyzed.

2.2 Aquatic ecosystems

Metals enter the aquatic environment vid industrial and domestic sewage discharges, dumping, surface run-off, atmospheric fallout etc.

2.2.1 Effects of metals

Metals - normal constituents of the aquatic environment as opposed to many other contaminants -are always present in at least trace amounts in the organisms which are adapted to natural variations in metal concentrations in food and water. Usually the natural background levels are extremely low and every increase through anthropogenic input can be expected to be harmful to the biota.

The aquatic environment is extremely variable and there are many factors which will modify the effects of a metal on the biota. Ionic strength is one variable - marine, brackish and freshwaters are obviously very different - but also pH, redox potential, hardness, the presence of organic and particulate matter etc, greatly influence both the chemical speciation of the metal, the type and stability of the ecosystem and thereby the impact of a metal. Generally, only the free metal ion is toxic whereas the portion bound in chelates and complexes or adsorbed onto particles is comparably inactive. Thus, many metals show a reduced toxicity in hard water and in water with a high content of organic or particulate matter. In acidified lakes, a larger portion of most metals is in free ionic form, rendering them more toxic than in a lake with neutral or basic water.

In the aquatic environment the most obvious ecological effects of metal pollution can be found in rivers and lakes receiving waste water and tailings from mines and from the processing of ores. Many rivers and lakes have parts where invertebrate fauna is lacking or markedly poor in variety and abundance and where the water is too toxic for survival of many fish species.

2.2.2 Uptake and accumulation

Environmental variables and chemical speciation also greatly influence the uptake and accumulation of a metal by an organism. For example, the arsenic levels in fresh water fish are lower than in marine fish. The concentrations have been found to be well correlated with salinity. Even the extremely high local contamination in the northern low-salinity part of the Baltic does not result in arsenic levels much higher than those commonly found in the oceans. Another example is the great difference between methylmercury - which is accumulated - and inorganic mercury - which is not.

Plankton, macroalgae and many species of molluscs do not seem to be able to regulate their uptake and/or output of metals. They can concentrate metals from the ambient water up to several thousand times. The passive nature of the uptake of cadmium, copper and zinc has been demonstrated for a brown algae species for which consistent concentration factors were obtained over a wide range of dissolved concentrations (Morris and Bale, 1975).

Cadmium and mercury (methylmercury) are accumulated in aquatic organisms to a very great extent and many other metals, e.g.

Pb and As, may be accumulated to a varying degree. It generally seems that the uptake from the ambient water makes a more significant contribution to the metal contents in aquatic organisms than a biomagnification via the food chain.

Bioaccumulation of lead has been studied in a river ecosystem (Leland and McNurney, 1974). Accumulation by benthic invertebrates and fishes was shown to be a function of niche and habitat. Lead did not seem to biomagnify in the food chains, detritus and herbivores having higher lead levels than the carnivores. Fagerström and Åsell (1973) employed a mathematical model to investigate the bioaccumulation of methylmercury in a three step fresh water food chain (bottom fauna - roach - northern pike). It was concluded that: 1: Bottom fauna, even when containing low amounts of methylmercury, may account for a significant fraction of the gross intake of methylmercury into the food chain; 2: The question of whether dietary or gill uptake is the more important can only be answered related to a specific system and trophic level. For the overall food chain, gill uptake is probably the most important route; 3: The top predator, northern pike, does not reach a steady state with respect to mercury in flesh during its lifetime; 4: If the rate of methylmercury supply to the food chain is instantaneously changed, the time to reach a new equilibrium with the environment may be long as 10 years in the top predator.

REFERENCES

Andersson, A. and Nilsson, K.O. (1972). Ambio 1, 176-179.

Benenati, F.E. and Risser, P.G. (1975). In: "International Conference on Heavy Metals in the Environment." p. C-154 (abstract only) Oct. 27-31, 1975, Toronto.

Bovay, E. (1971). In: "Bulletin des Eidgenössische Gesundheitsamtes." Beilage B. No. 3. Zurich (In French).

Ducker, H. (1976). Personal communication.

Fager, E.W. (1972). Amer Nat. 106, 293-310.

Fagerström, T. and Åsell, B. (1973). Ambio 2, 164-171.

Fagerström, T. and Åsell, B. (1974). In: "Proceedings of the International Conference on Transport of Persistent Chemicals in Aquatic Ecosystems." pp IV:11-16, May 1-3, 1974, Ottawa.

Goodman, G.T. and Roberts, T.M. (1971). Nature 231, 287-292.

Holzman, R.B.C. (1968). In: "Radiation Protection Pt 2." (W.S. Snyder, ed) pp 1087-1096. Pergamon Press, London.

Hutchinson, T. (1975). In: "International Conference on Heavy Metals in the Environment." p. C-316 (abstract only) Oct. 27-31, 1975, Toronto.

Jernelöv, A. (1974). In: "Conference on Intoxication due to Alkyl Mercury Treated Seed, Baghdad, Iraq." Nov. (Abstracts published by Swed. Wat. Air Pollut. Res. Lab., Stockholm).

John, M.K. (1975). In: "International Conference on Heavy Metals in the Environment." p. C-233 (abstract only) Oct. 27-31, 1975, Toronto.

Leland, H.V. and McNurney, J. (1974). In: "Proceedings of the International Conference on Transport of Persistent Chemicals in Aquatic Ecosystems." pp III-17-23, May 1-3, 1974, Ottawa.

Lundström, H. (1971). "Environmental Research." National Swedish Environment Protection Board. (In Swedish).

Morris, A.W. and Bale, A.J. (1975). Estuarine Coastal Marine Sci. 3, 153.

Peterson, P.J. (1971). Sci. Prog. Oxf. 59, 505-526.

Pielou, E.C. (1969). "An Introduction to Mathematical Ecology." Wiley-Inter Science, New York, VIII + 286 pp.

Schroeder, H.A. and Balassa, J.J. (1961). J. Chron. Dis. 14, 236-258.

Tyler, G. (1975). In: "International Conference on Heavy Metals in the Environment." p. C-79 (abstract only) Oct. 27-31, 1975, Toronto.

Webber, L.R. and Beauchamp, E.G. (1975). In: "International Conference on Heavy Metals in the Environment." p. C-60 (abstract only) Oct. 27-31, 1975, Toronto.

Westermark, T., Forberg, S. and Odsjö, T. (1973). "Preliminary Studies on the Occurrence of Mercury, Indium, Cadmium and Arsenic in Plants and Animals in the Vicinity of a Smelter." Report from the Museum of Natural History, Stockholm. (In Swedish).

Westermark, T. and Sjöstrand, B. (1972). Adv. Activat. Anal. 2, 57-88.

Whitby, L.M. (1975). In: "International Conference on Heavy Metals in the Environment." p. C-121 (abstract only) Oct. 27-31, 1975, Toronto.

STANDARDS AND CRITERIA

Lars Friberg and Velimir Vouk

There is a need for administrative regulations to prevent adverse health effects among people exposed to chemical or physical agents in the general as well as the industrial environment. Such regulations may have different legal implications in different countries. For the time being they will be referred to as standards.

The aim of environmental health standards is to protect individuals, human populations and their progeny from the adverse effects of hazardous environmental factors, including chemicals.

A sound principle of health protection is to keep all exposures as low as reasonably achievable, subject to the conditions that the appropriate exposure limits, defined by the standard, are not exceeded. The relationships between human disease and exposure to chemicals are complex and poorly understood. Death and disease are only the extreme end of a spectrum of biological changes which include a variety of "adverse" and "non-adverse" effects.

Environmental health standards for chemicals may be formulated either in terms of concentrations in environmental components (examples are air quality standards, "threshold limit values" and drinking water standards) or in terms of amounts of substances that may be taken into the body within an appropriate unit of time (e.g. acceptable daily intake). These concentrations and amounts should be sufficiently low so that the threshold dose (if it exists and can be determined) will not be reached, or so that the population of concern will not be subjected to "unacceptable risk" even following life-time or working life-time exposure. In some cases, as for irritant air pollutants, the distribution of exposure concentrations in time should also be considered.

Preceding page blank

Standards may be also emission standards or effluent standards, defining permissible concentrations or amounts of a substance in stack gases or industrial or municipal waste waters. Setting up emission standards may be one way to arrive at permissible concentrations of toxic metals in fuels, for example, coal and oil. Standards may limit the concentration of toxic substances in products used on a large scale, for example lead compounds in paints, or they may prescribe the quantity of a substance to be used, handled or transported at any time, and the manner of its use, handling or disposal.

It is obvious that the standard setting process will necessarily involve many considerations besides toxicology. Social, cultural and economic considerations should be taken into account in setting standards, but never to the detriment of health protection which should be of primary concern. The standard setting process is often very different in different countries and different types of society, and with few exceptions, such as food standards, there are no international agreements on standards for chemical substances. In general, however, it involves appraisal of toxicological data, particularly of dose-response relationships, and of information on the effects on non-human targets (plants, animals, materials); social and economic analysis, policy analysis and review of experience elsewhere, leading eventually to an administrative or policy decision concerning the standard. Other relevant questions include the technological feasibility of achieving a standard, cost and benefit of implementing it, means of enforcement, other public health priorities, etc. Many of these topics are outside the scope of the present discussion.

Standards are necessary for toxic metals, often more so than for other chemical substances. Metals are not disintegrated in nature and may be transported over great distances. Furthermore, they have a tendency towards accumulation in nature as well as in the human organism.

Theoretically an ultimate goal might be to ban the use of highly toxic metals. For several reasons this is not possible. Metals are indispensable in a technological society. Furthermore, highly toxic metals often occur together with nontoxic metals in nature. Cadmium, for example, is found together with zinc in a certain proportion in ores and it would be impossible to ban cadmium without taking severe measures concerning the use of zinc as well. Some metal compounds have been successfully prohibited under certain circumstances, however. Several governments have, for example, banned the use of methylmercury as a fungicide. In some countries, the use of tetra ethyl lead as an additive to motor gasoline, previously widespread, has now been forbidden. Generally, there is a tendency to reduce the concentrations of lead in gasoline by means of legislative measures. Cadmium and lead have been widely used as color pigments. In several countries severe restrictions on this practice are now being enforced.

Thus, with a few exceptions we have to live with some exposure to metals both industrially and in the general environment. A major problem will be to define which exposure can be accepted with negligible risks or with only a minor, defined risk. Such risk evaluations may refer to effects on the environment as well as on humans. The present discussion will focus on the effects on humans only. Basically, however, the same principles apply when evaluating effects on the environment. For certain metals such risks may be the critical ones, meaning that they should receive priority from the standpoint of prevention and control.

There are different ways to control exposure. One approach is called the best practical means approach while another focusses on limiting concentrations known or suspected to be associated with health effects.

The best practical means approach implies that an industry installs the best available technique to reduce emission of hazardous substances, taking into consideration, however, technical and economical aspects. What is meant by "best practical means" is often a question of debate. This has to do with economic considerations and the priorities given to different forms of health protection. It also has to do with differences in philosophy of risk acceptance.

If the best practical means approach is used in a proper way it brings the advantage that resulting concentrations in different media, e.g. in ambient air and soil, around a point source will be as low as possible with the available cleaning technique. Under certain circumstances this might mean that e.g. ambient air concentrations will be considerably lower than would be necessary from the toxicological point of view. Large safety margins may thus sometimes be achieved. The approach has obvious drawbacks as it does not take into consideration, at least not explicitly, information on dose-effect and dose-response relations for hazardous substances. There is thus no assurance that the best practical means approach will bring hazardous substances in the different media down to levels which can be considered acceptable from the toxicological and epidemiological viewpoint.

Standards based on health effect evaluations have in principle existed for many years due to the efforts of several organizations and regulatory agencies. Well-known is the work carried out in the USA by a private group belonging to the Association of Governmental Industrial Hygienists. Threshold limit values recommended by this group certainly have been of value both nationally and internationally in the absence of more detailed evaluations. During recent years agencies like US Environmental Protection Agency and US National Institute of Occupational Safety and Health have embarked on large programs for working out criteria documents. These are basically an evaluation of dose-effect and dose-response data for different substances, including metals.

The World Health Organization has given considerable attention to standards and criteria for the quality of the environment during the last two decades. The work has involved drinking water, food additives and contaminants, pesticide residues, carcinogenic risks of chemicals, and air quality. In 1973 the World Health Organization embarked on an internationally coordinated program on the assessment of health effects of environmental conditions. This program, in which actively participate more than 20 WHO member states, is called the WHO Environmental Health Criteria Programme and was initiated in response to a number of World Health Assembly resolutions. It also took into consideration the relevant recommendations of the UN Conference on the Human Environment in Stockholm, 1972, and of the Governing Council of the United Nations Environment Programme (WHO, 1976).

When selecting priorities of the program and preparing criteria documents the following considerations are taken into account:

a) severity of adverse effects on the population, in particular, whether there are irreversible or chronic effects, adverse genetic implications, or embryotoxic or teratogenic effects;

b) ubiquity and abundance of the agent in man's environment, particularly those occurring naturally or produced inadvertently;

c) persistence of the agent in the environment, resistance to environmental degradation and accumulation in man or in food chains;

d) possibility of metabolic degradation or synthesis in biological systems which may produce metabolites either more or less toxic than the parent compound;

e) size, type and demographic characteristics of exposed populations; the frequency and magnitude of exposure particularly of highly vulnerable groups of the population.

The full list of priorities for the criteria documents embraces some 70 chemicals and/or classes of chemicals as well as physical hazards. The different criteria documents are now in different stages of progress. Criteria documents for some metals, such as mercury and lead have already been published (WHO, 1976, 1977). For other metals the editing is almost complete and publication will take place during 1978. This applies to cadmium, manganese, tin and organotin compounds and titanium. For certain of the substances, e.g. cadmium, lead, and mercury, it has been decided to have a reevaluation within the next three years.

One of the main objectives of the WHO Environmental Health Criteria Programme is to assess existing information on the relationship between exposure to environmental pollutants (or other physical and chemical factors) and human health, and to provide guidelines for setting exposure limits consistent with health protection, i.e. to compile environmental health criteria documents. Of importance is that the assessments referred to should be made in relation to man's total exposure to a given agent, from different media or conditions, for example, food, air, water, work and the home. In the past, when evaluating risks, the total exposure was not generally considered. The inadequacy of this approach was apparent for pollutants that may reach man by several pathways as is the case with lead, cadmium and some other metals and certain persistent organic compounds.

Cadmium, for example, accumulates in the body over the entire life span. The most important exposure to cadmium for those not industrially exposed is through food, which in turn has been contaminated through deposition of cadmium from the ambient air or the use of cadmium-containing fertilizers, including sludge. For smokers, however, the smoking of 20 cigarettes a day will alone give rise to an accumulation of cadmium in the body comparable with the accumulation from cadmium in food. For workers in certain industries the most important exposure will be from cadmium in workroom air.

The developing of a criteria document for a substance means evaluation of dose-effect and dose-response relationships. Dose-effect relationships indicate how an effect may increase in severity with increasing exposure. A dose-response relationship shows how the number of persons afflicted varies with increasing exposure (see Chapter 00).

In evaluating dose-response curves for chemical substances, including metals, it is usually implied that there is a threshold below which no adverse effects will occur. On purely statistical grounds it is as a rule not possible to determine with any degree of certainty a threshold, i.e. a no-effect level for humans. One reason is the great variability of susceptibilities (tolerances) of individuals in human populations, and the size of populations at risk which are often very large compared with the populations on which the information on health effects has been obtained. Furthermore, no-effect level may vary depending on the conditions of exposure and on the presence or absence of various factors that may modify the response (e.g. other chemicals, heat, radiation, etc.). For this reason no-observed-effect levels are divided by safety factors.

Most regulatory authorities rely on the use of safety factors but there are no precise guidelines for deciding the appropriate size for such factors. In general, the size of the safety factor will depend on (a) the nature of toxic effects, (b) the size and type of population to be protected, and (c) the quality of toxicological information available. A factor of 2-5 may be considered as sufficient if the effect against which protection is desirable for individuals or a population is, for example, lung or skin irritation, if only a small number of workers are likely to be exposed and if the toxicological information is derived from human data. On the contrary a safety factor as large as 5,000 may be required if the possible effect is very serious (e.g. cancer), if the general population is to be protected, and if the toxicological data are derived from limited experiments on laboratory animals.

In some cases, the safety factor may be a value that has been used with reasonable success and is, therefore, perpetuated.

In developing rules for protection against ionizing radiation, the assumption has been made that thresholds do not exist and that any exposure may involve some degree of risk. This philosophy has nowadays been adapted in many quarters also for carcinogenic and mutagenic effects of chemical substances, including metals. The question of the form of the dose-response curve for carcinogenic substances was discussed in detail at an international symposium at the Karolinska Institute in Stockholm, March 1977 (Task Group on Air Pollution and Cancer, 1978). The meeting was arranged to evaluate risks of cancer from carcinogenic substances, particularly substances in the ambient air derived from a combustion of fossil fuels. The conclusions reached, however, have implications for the evaluation of carcinogenic risks more generally, in the general as well as in the industrial environment.

One important conclusion reached with regard to risk assessment methodology for carcinogenic substances was, "In considering protection of human populations, and in the absence of firm evidence to the contrary, it is not justified to assume a "threshold", i.e. that there is a dose below which no response is obtained." It was also stated, "In the absence of relevant dose-response data the most appropriate way to estimate the risk of lung cancer is to assume that it will be directly proportional to the increase in dose. For small added doses, a simple linear dose-response curve, as used in radiation carcinogenesis, is appropriate." The conclusions mean that basically the same philosophy was adopted as the one long used in radiation protection, namely the assumption that thresholds do not exist and that any exposure may involve some degree of risk. Similarities with ionizing radiation were considered as best founded in regard to direct (ultimate) carcinogens (not requiring metabolic activation) acting on directly exposed tissues.

The conclusions of the Karolinska Institute symposium also mean that, one could apply the concept of collective dose in risk assessment relative to carcinogenic substances that are persistent in the environment and which are ultimate carcinogens (direct carcinogens), e.g. metals. The collective dose can be defined as the product of the average dose to an individual in a defined exposed group, called "per caput" dose, and the number of individuals in that group (ICRP, 1977). The use of the collective dose (i.e. the simple summation of doses) in the evaluation of the total risk to a population is valid only under the assumption that there within the range of exposure conditions considered is a linear relationship without threshold between dose and the probability of an effect. In radiation protection, the collective dose is expressed in man.rad units. For chemical carcinogenesis, the unit could be man.mg/kg or in case of carcinogenic air pollutants man.ng/m^3 (if it is assumed that dose is proportional to the concentration of a carcinogen in air).

If the exposure is extended in time, the concept of dose commitment becomes useful. The dose commitment is defined as the time integral of the per caput dose rate. The collective dose commitment is obtained by integration of the collective dose rate. For chemicals with a tendency to accumulate in the organism and in the environment, such as cadmium, the dose commitment approach might be an appropriate way of assessing the risk from a practice leading to the release of the substance into the environment.

Complete information concerning dose-effect and dose-response relationships for humans is not available for any single metal. It is therefore necessary to rely on scanty data when assessing health risk. Sometimes there are no data on humans at all and animal data must suffice. This makes every evaluation all the more complicated. Animals may react quite differently, partly in terms of their sensitivity to a particular substance and partly in terms of their metabolism of the substance. This may refer to deposition as well as absorption, biotransformation, accumulation and excretion of the substance. As an example, it can be mentioned that methylmercury is a very toxic form of mercury in humans. It is highly stable and is accumulated in the body as methylmercury. In rats, this compound is readily broken down into inorganic mercury and the effects are less severe.

What has been said does not imply that animal experiments are of no value. For studying mechanisms of action the opposite is definitely true. When screening for potentially carcinogenic or mutagenic substances, animal experiments are of tremendous value. One has to be aware of the limitations of the methods, however, and also appreciate that it is very seldom possible for an animal experiment to reproduce a real-life human exposure situation. Animal experiments will have to be used as a basis of risk assessment in the case of new chemicals including, e.g. organometallic compounds, for which there are no data regarding human exposure.

For metals very few published, long-term studies on humans are available, in which reliable exposure data are reported in conjunction with observed effects. There are more studies where effects and response have been related to concentrations of metals in some biological indicator media, e.g. blood or urine. Such evaluations have the advantage of allowing the use of information from other exposure situations than those related to inhalation. For example, most information of importance for environmental health criteria for mercury and cadmium comes from studies where humans have been exposed to the metal through contaminated food.

A prerequisite for using data on dose-response relationships based on concentrations in indicator media and resulting health effects is knowledge of the metabolic model for the metal. This means the knowledge of parameters related to absorption, biotransformation, distribution, accumulation and excretion of the substance. For certain substances the metabolic model is well defined and simple. Methylmercury is absorbed to almost 100% whether ingested or inhaled and the accumulation within the body follows a one-compartmental model with an average biological half-time of about 70 days. If the daily exposure of the metal is known, it is therefore possible to use simple equations to estimate the accumulation of mercury. Data from epidemics of mercury poisoning in Japan and in Iraq have been reasonably good as far as elucidating associations between concentrations in indicator media (hair and blood) and effect and response is concerned. Based on such data and the metabolic model it has been possible to come up with environmental health criteria for methylmercury for ambient air, industrial air and food.

For cadmium, data based on concentration of cadmium in kidneys, together with a metabolic model, have been of value in calculating acceptable concentrations in air, food and water. The metabolic model for cadmium is not as well-known as would be desirable, however, and environmental health criteria based on the use of such a model are therefore somewhat more uncertain.

As mentioned in the introduction, standards as a rule refer to concentrations in different environmental media, e.g. ambient air, food, water, and industrial air. There is a need also to come up with standards for metals in biological media, like blood, hair, and urine. In practice such standards are often used, e.g. lead in blood, cadmium in urine and mercury in blood or hair. Before setting such standards it is imperative to examine in detail what the indicator values really mean. For certain substances such values may be of no assistance at all; for others they may reflect recent exposure, body burden and concentrations in critical organs.

For methylmercury there are definite possibilities of using concentrations of mercury in blood or hair. As mentioned, such concentrations and knowledge of the metabolic model have been the starting point for evaluating risks from exposure via air as well as food. For cadmium the situation is much more difficult. During a long-term low-level exposure urinary cadmium values are related to concentrations in kidney and total body burden. On the other hand high urinary cadmium values may be found when kidney dysfunction has occurred. High cadmium levels may therefore be a result of cadmium intoxication. Cadmium values in blood will first of all reflect recent exposure, perhaps the exposure during the last few weeks. As such, blood values may be of substantial value. If exposure ceases, however, cadmium concentrations in blood will decrease more rapidly than the cadmium level in the body as a whole. One may then find low blood values even though the concentrations in the critical organs, the kidneys, could well be high.

The situation for lead is also complicated. Most of the absorbed lead, about 90%, is found in the skeleton, where it is in an inert form. The lead that constitutes the direct danger to health is in the blood and bone marrow. As the biological half-time in blood is about 20 days, while the half-time in the bones may be 10-20 years, it is easily understood that blood concentrations do not reflect the accumulated lead. Even so, the monitoring of blood levels is of great value in preventing clinical effects because of the known relation between concentrations in blood and effects. Unfortunately there are not very many data which show how air lead levels relate to blood lead levels despite the several years of industrial monitoring of both air and blood.

The work within the WHO Environmental Health Criteria Programme has clearly shown that substantial information is available for several metals. It is also obvious, however, that available data from toxicological and epidemiological studies do not necessarily contain information which would make it possible to come up with a detailed evaluation of dose-effect and dose-response relationships. There is an obvious need for more intense research directed towards information needed for a health risk evaluation. Furthermore, it seems to be extremely important that information on studies carried out be made available to a greater extent than is now the practice. This has been pointed out explicitly by the Task Group on Metal Accumulation (1973) and The Task Group on Metal Toxicity (1976). The Task Group on Metal Accumulation said, "There is a lack of epidemiological data on the parameters discussed above in populations with industrial exposure. This lack is even more striking in relation to the growing number of such studies on populations with general environmental exposure, which is usually at a much lower level. There is a need in industrially exposed populations for standardized, wherever possible collaborative, epidemiological studies, where cohorts can be followed in time and where groups can be related to each other. With some occupational exposures to the less common metals, only small groups may be available for study in any one country, so that international collaboration in epidemiological studies would again be of value."

REFERENCES

ICRP (1977). Radiation Protection, Recommendations of the International Commission on Radiological Protection, ICRP Publication 26 (In press).

Task Group on Air Pollution and Cancer - Risk Assessment Methodology and Epidemiological Evidence. (1978). Report from an International Symposium at the Karolinska Institute, Stockholm, 1977. Environ. Health Perspect. (In press, 1978).

Task Group on Metal Accumulation (1973). Environ. Physiol. Biochem. 3, 65-107.

Task Group on Metal Toxicity (1976). In: "Effects and Dose-Response Relationships of Toxic Metals." (G.F. Nordberg, ed) pp 1-111. Elsevier, Amsterdam.

WHO (1976). Background and purpose of the WHO Environmental Health Criteria Programme. In: "Environmental Health Criteria 1: Mercury." pp 5-14.

WHO (1977). "Environmental Health Criteria 3: Lead." pp 3-160.

CLINICAL EFFECTS, DIAGNOSIS AND TREATMENT OF METAL POISONING - GENERAL CONSIDERATIONS

George Kazantzis

1. Clinical effects

1.1 General considerations

Clinical effects following the absorption of a toxic metal are manifested by abnormal signs accompanied by symptoms, and this pattern of signs and symptoms may comprise a syndrome. A syndrome may be acute, terminating in death or followed by partial or complete recovery. It may be subacute in onset or there may be a longer latent interval between absorption and its development, which may then follow the same course as above. Such a syndrome is usually referred to as a "poisoning", it being customary, for example, to refer to "heavy metal poisoning". However, toxic metals produce other important clinical effects not usually included under this term. These effects are (i) hypersensitivity reactions which may involve the skin, lung, kidney, hemopoietic system and possibly the nervous system; (ii) the induction of cancer after an appropriate latent interval; and (iii) teratogenic malformations. Furthermore, an increasing number of metals are being shown to give rise to genetic damage in test systems and the question arises as to whether such damage may also occur in man, with a consequent increase in abnormal individuals in later generations. These other effects, because of their importance and magnitude, are considered elsewhere.

Adverse effects, which may be manifest by biochemical or physiological tests or even by abnormal clinical signs, may be present in the absence of symptoms or in the presence of vague, ill defined complaints of ill health which do not comprise a recognizable syndrome. Common examples are anemia following exposure to lead compounds and renal tubular dysfunction following long-term exposure to cadmium.

The clinical effects described above imply that an adverse biological change has been produced in the organism with some impairment of cellular function resulting from exposure. The model on which this adverse effect is based assumes that a critical concentration of the toxic metal has been attained in the critical organ to give rise to a critical effect. This critical effect may or may not be of immediate importance to the health of the organism as a whole (Nordberg, 1976). It may be argued that such a critical effect cannot be considered to be synonymous with a clinical effect. Here it is useful to distinguish between critical and subcritical effects. Effects may also occur below the critical concentration in the critical organ which do not impair or appear to impair cellular function (Nordberg, 1976). While the biological significance of such a subcritical effect may be unknown, it would be reasonable, given the existing state of knowledge, to exclude such a subcritical effect from clinical consideration.

1.2 Exposure pattern and clinical effect

A number of factors act as determinants of clinical effect following exposure to a toxic metal. Such factors include dose; route of absorption; the chemical and physical form ot the metal concerned; genetic variation manifested through racial, familial and individual susceptibility; dietary pattern and nutritional status; immunological status; presence of intercurrent disease and interaction with other chemical compounds in the environment. Some of these determinants are considered in the chapter on Factors modifying dose-effect and dose-response relationships in this volume and in Volume II of this series, on Specific metals. The exposure pattern, in terms of concentration, time and route of exposure is an important determinant of clinical effects which required further consideration here.

A short-term exposure may produce a very different clinical effect from a similar exposure in terms of total dose over a longer period of time. The effects of ingestion of a toxic metal are more often seen in the domestic or general environment than in an industrial setting. Short-term, high-level exposure by ingestion may follow the accidental, suicidal or homicidal ingestion of a toxic metal compound, giving rise to well recognized acute syndromes, usually involving the gastrointestinal tract, but which may involve secondarily the renal, cardiovascular, nervous and hemopoietic systems. Long-term, low-level exposure by ingestion is seen increasingly in the general environment as a result of the contamination of food and drink by metals which have cumulative properties in the organism. Clinical effects may involve any organ system in the body, but the gastrointestinal tract is not primarily involved.

By contrast, short-term, high-level inhalation exposure is most often occupational in origin. It may give rise not only to acute respiratory effects, but may also involve the cardiovascular, central nervous, renal and hemopoietic systems. Again, long-term, low-level inhalation exposure is usually occupational in origin and its control forms a large part of industrial hygiene practice. However, long-term, low-level inhalational exposure to certain toxic metals may also occur in the general environment and from cigarette smoking. The effects may involve any organ system in the body and may spare the respiratory system.

Mercury forms a good example of the extreme variation in clinical effect which may be produced depending on the pattern of exposure and the chemical form of the metal. The lung is the critical organ following short-term, high-level inhalation exposure to mercury vapor, resulting in pneumonitis and respiratory failure, while the central nervous system is the critical organ following long-term exposure to mercury vapor. Following the ingestion of an inorganic soluble mercuric compound, the kidney is the critical organ, manifesting with anuria due to tubular necrosis. As a result of the long-term ingestion of methylmercury as a food contaminant, nervous system effects may develop but with a different clinical syndrome to that seen after the long-term inhalation of inorganic mercury vapor.

The variability in clinical effect produced by the toxic metals is further illustrated in the following section.

1.3 Acute clinical effects of metals

1.3.1 Gastrointestinal effects

Acute gastroenteritis follows the ingestion of a sufficient quantity of most metals in the form of soluble salts. A not uncommon occurrence is the contamination of food or drink, especially if acidic, by dissolution of metal from food containers. Symptoms develop a short time after ingestion, often involve a number of people partaking of the meal and may be labelled as "food poisoning". Vomiting and diarrhea may be followed by circulatory collapse and involvement of other systems depending on the poison absorbed. Poisoning with soluble compounds of antimony, cadmium, copper, lead, tin and zinc has occurred in this way. Acute gastroenteritis with collapse may be the predominant features following the ingestion of rodenticides containing arsenic, barium or thallium, yellow phosphorus or zinc phosphide. Similar symptoms may follow the ingestion of soluble compounds of bismuth, chromium, iron, silver and vanadium. The ingestion of a soluble mercuric salt gives rise to a gastroenteritis with a bloody diarrhea which may resemble fulminating ulcerative colitis. Lead colic has on many occasions simulated an acute surgical emergency resulting in an unnecessary laparotomy.

1.3.2 Respiratory effects

Acute chemical pneumonitis which may be accompanied by pulmonary edema follows the inhalation of a number of freshly formed metal fumes. Particularly toxic in this respect is the inhalation of freshly formed cadmium oxide fume, acute symptoms developing some hours after an apparently innocuous exposure (Beton et al., 1966). The inhalation of antimony pentachloride, arsine, beryllium fume, iron pentacarbonyl, lithium hydride, nickel carbonyl, titanium tetrachloride, selenium dioxide, hydrogen selenide, vanadium pentoxide or zinc chloride can give rise to a similarly acute picture with pulmonary edema. The inhalation of high concentrations of mercury vapor or dust or inorganic mercury compounds can also give rise to pneumonitis before other symptoms of mercurialism develop (Teng and Brennan, 1959). Respiratory symptoms with rigors and fever resembling an acute respiratory infection may follow the inhalation of freshly formed zinc fume, brass fume or other metallic oxides, giving rise to metal fume fever (Hunter, 1975). Pneumonic consolidation has followed the inhalation of manganese dust. The inhalation of complex salts of platinum can give rise to acute and severe asthma. Decomposition products or other contaminants of industrial air may be responsible for some of these effects.

1.3.3 Cardiovascular effects

A number of metallic ions interfere with the normal function of myocardial cells giving rise to arrhythmias including ventricular fibrillation. Fibrillation may be responsible for a fatal termination in poisoning by antimony, barium or lithium salts. Cobalt can give rise to cardiomyopathy (Alexander, 1972). Some metals have been shown to have a hypotensive effect. These include antimony, cadmium, cobalt, copper, iron and vanadium, a state of shock being a common presenting feature in poisoning with these metals.

1.3.4 Effects on the central nervous system

Metal poisoning may present with an acute illness involving the central nervous system. Most important, because still unfortunately not uncommon in children, are convulsive attacks which may terminate in coma or death as a result of acute lead poisoning. A successful therapeutic outcome in such a case is dependent on early diagnosis and treatment. Convulsions may also follow the absorption of iron, barium, lithium, thallium and organic tin compounds. An acute psychosis may also be the presenting feature in metal poisoning. Following heavy exposure to tetraethyllead a patient may present with delusions, hallucinations and hyperactivity which may precede coma and death (Beattie et al., 1972).

1.3.5 Renal effects

Renal damage, manifesting as oliguria or anuria due to acute tubular necrosis is another way in which metal poisoning may present, although this feature often follows an initial presentation with acute gastrointestinal, circulatory or respiratory effects. Oliguria and anuria due to tubular necrosis are common occurrences especially in children, following the ingestion of soluble mercuric or iron salts. The condition may also follow pneumonitis resulting from cadmium fume inhalation (Beton et al., 1966). Renal failure has been a sequel to the absorption of a number of soluble metal compounds including antimony, arsine, bismuth, copper, uranium and vanadium salts, particularly if there is associated short or intense renal vasoconstriction.

1.3.6 Hemopoietic effects

An acute hemolytic anemia often accompanied by renal failure may be the presenting feature following the inhalation of arsine or of stibine gas (Jenkins et al., 1965). Acute hemolysis has also followed the ingestion of large doses of soluble copper salts.

1.4 Chronic clinical effects of metal toxicity

The term chronic effect is a relative one, signifying that the clinical effect may develop gradually and may persist for a longer interval than an acute effect. Chronic effects are associated with longer term exposure at lower levels than acute effects. Only examples of chronic effects will be given here, the great variety of these being described in the individual metal chapters.

1.4.1 Chronic gastrointestinal effects

Some chronic gastrointestinal effects may in fact be examples of mild acute poisoning following the repeated ingestion of the toxic metal over a period of time. Thus prolonged diarrhea with weight loss occurred in a child whose drinking water was heavily contaminated with copper. Similarly chronic gastrointestinal symptoms have occurred in persons ingesting canned juice contaminated with high concentrations of tin or of zinc. However intestinal colic has been observed in industrial workers and in children with relatively low-level lead exposure, and this can be considered a true example of chronic lead poisoning (Beritic, 1971). Anorexia, nausea, diarrhea or constipation with weight loss are seen in chronic arsenic poisoning but are usually accompanied by neurological and skin involvement. Anorexia, nausea and vomiting in industrial workers may be seen in selenium and in tellurium poisoning, when the characteristic garlic odor of the breath may also be elicited. Anorexia and often digestive disturbances have also been reported in people living in seleniferous regions with a high dietary intake of selenium. Anorexia, nausea, vomiting and diarrhea followed by constipation accompanied by a burning sensation of the tongue and stomatitis have resulted from occupational exposure to thallium compounds.

1.4.2 Chronic hepatic effects

A number of metals are hepatotoxins, giving rise to effects ranging from abnormalities in hepatic enzyme levels to clinical jaundice. Such effects have been reported following exposure to antimony, arsenic, bismuth, copper, chromium, iron, manganese and selenium.

1.4.3 Chronic respiratory effects

Chronic pulmonary disorders giving rise to dyspnea may result from absorption of metal dust or fume. An inflammatory response consisting of noncaseating granuloma formation followed by pulmonary fibrosis may result from exposure to beryllium after a latent interval of many years (Stoeckle et al., 1969). Progressive dyspnea with the clinical, radiological and functional changes associated with emphysema is seen in workers exposed to cadmium oxide fume or dust. The respiratory abnormality is usually, but not invariably, associated with renal tubular dysfunction. Pulmonary fibrosis has followed occupational exposure to finely powdered aluminum metal dust. Dyspnea, wheeze and productive cough giving rise to an illness resembling asthmatic bronchitis have followed occupational exposure to vanadium pentoxide dust. Progressive breathlessness associated with pulmonary fibrosis has occurred in hard metal workers, exposed to the dusts of tungsten and titanium carbides with cobalt used as a binder. Chronic asthma can occur in workers sensitized to chromium, following the inhalation of chromate dust or chromic acid fume and also to organic platinum compounds. Once sensitization to platinum has occurred, the reaction can be triggered by exposure to minute quantities of the salts of chloroplatinic acid.

1.4.4 Chronic effects on the nervous system

Peripheral neuropathy may develop in the recovery stage of acute arsenic intoxication, about one to three weeks after exposure. It is a mixed motor and sensory neuropathy, with a "glove and stocking" distribution. Neuropathy develops in those who survive the acute gastrointestinal effects of thallium poisoning and may lead to a later fatal termination. With both these metals skin changes occur at a later stage, with the former metal, arsenical pigmentation, and with the latter, hair loss. A motor neuropathy involving predominantly the upper limbs with wrist drop and extensive weakness of the fingers is seen in chronic lead poisoning. By contrast, antimony salts of organic acids give rise to a sensory neuropathy which may involve the trigeminal nerve. Bismuth and copper have also given rise to peripheral neuropathy.

Permanent brain damage with cerebral cortical atrophy or hydrocephalus may be the sequel to acute lead encephalopathy. Convulsions may recur over a long period, and idiocy may develop. The complex relationship between moderately elevated body burdens of lead in childhood and certain functional neurological disorders is considered in the chapter on lead (see also Landrigan et al., 1975).

Degenerative changes in the nerve cells of the basal ganglia giving rise to a parkinsonian syndrome result from the absorption of manganese following long-term occupational exposure (Mena et al., 1970).

Degenerative changes affecting in particular the granular cells in the cerebellum and neurones in the calcarine, pre- and post-central cortex, follow the absorption of alkylmercury compounds and present with a characteristic neurological syndrome whose principal features are paresthesia of extremities and face, ataxia, dysarthria and concentric constriction of the fields of vision. Pyramidal signs may also occur. Another characteristic neurological disorder consisting principally of intention tremor of the hands, tremor of the eyelids and tongue and a combination of behavioral and personality changes known as erethism develops after chronic exposure to mercury vapor.

1.4.5 Chronic renal effects

Certain chronic renal disorders may also follow exposure to toxic metals. Proximal renal tubular dysfunction consisting principally of tubular proteinuria, glucosuria, aminoaciduria and phosphaturia may develop following cumulative exposure to cadmium, lead and uranium compounds. Hypercalciuria has also occurred in chronic cadmium poisoning as a further manifestation of renal tubular dysfunction and this has led to renal stone formation and to osteomalacia in a few cases following industrial exposure (Kazantzis, 1975).

Osteomalacia has been observed in a population environmentally exposed to cadmium in Japan. Although the skeletal disorder (Itai-Itai disease) only occurred in postmenopausal women, evidence of renal tubular dysfunction was found in the population as a whole (Friberg et al., 1974).

Progressive impairment in renal function terminating in uremia and indistinguishable clinically from chronic nephritis has been observed following childhood lead poisoning. The wide range in abnormal response of the kidney to absorbed metals can be further illustrated by reference to the nephrotic syndrome. Heavy proteinuria with hypoproteinemia and edema has followed exposure to inorganic mercury, organic gold and bismuth preparations.

1.4.6 Chronic hemopoietic effects

Chronic arsenic poisoning is associated with an anemia due to decreased red blood cell formation with increased destruction. The anemia of chronic lead poisoning also results from decreased hemopoiesis combined with increased red cell destruction (Goyer and Rhyne, 1973). By contrast, cobalt has an increased hemopoietic effect, and has given rise to polycythemia, but not to increased production of other cellular elements in the blood.

2. The diagnosis of metal poisoning

As can be seen from the above paragraphs, metal poisoning may involve any of the organ systems of the body and give rise to a wide variety of effects. The presenting features may be entirely non-specific, the clinical examination giving no lead on the cause of the illness. The principal features which should be considered in arriving at a correct diagnosis will now be elaborated upon.

2.1 The history of exposure

In most cases a history of exposure to a toxic metal will give the necessary clue. In an industrial situation there may be a clear history of exposure which may be obtained from patient, relative or co-worker. The clinician should not fail to take a full and accurate occupational history where a poisoning of occupational origin is suspected. It may be necessary to seek additional information from the employer or his agent, from an occupational hygienist or from a health and safety representative. Exposure to a toxic metal may however occur without this being suspected by any of the persons questioned, and this can only be inferred by an adequate knowledge of the work process involved. A good example may be given with exposure to arsine gas, which usually presents as an acute medical emergency. Arsenic may be a contaminant in scrap metals, flue dusts etc. and arsine may be formed in any reducing situation, or when a metallic dross containing arsenic comes into contact with water. The condition should therefore be suspected in a scrap metal worker presenting with the appropriate clinical features.

In the domestic environment an appropriate history may give an exposure inferred from medicaments such as iron pills or household chemicals. Unlabelled pills or chemicals should, of course, be kept for analysis. In a homicidal instance, a psychotic factory worker succeeded in poisoning three fellow workers in succession by adding a thallium salt to tea, causing the deaths of two men before the condition was suspected (Cavanagh et al., 1974). In the general environment diagnosis of metal poisoning, especially if caused by long-term absorption of a cumulative metal in the body, can be very difficult. Methylmercury poisoning in the environs of Minamata Bay took more than five years to diagnose (Kazantzis, 1971). However, methylmercury poisoning following the eating of contaminated bread in Iraq was diagnosed at the beginning of the outbreak, as the medical officers had previous experience of the condition.

2.2 The clinical features

The presenting clinical features, especially in acute poisoning, may be entirely non-specific. Lead colic has been repeatedly mistaken for the acute surgical abdomen, and ~~eyanid~~craniotomy has been performed following encephalopathy due to lead. Such mistakes would not have been made if a careful history had been taken. Certain clinical sequences should alert the physician to the possibility of metal poisoning. Gastroenteritis followed by peripheral neuropathy which is in turn followed by hairfall is a classical presentation for thallium poisoning. Gastroenteritis followed by oliguria or anuria follows the ingestion of inorganic mercuric and other soluble metal compounds as mentioned in the previous section. A hemorrhagic gastroenteritis, especially if accompanied by collapse and hypotension, should alert the clinician to an acute metal poisoning by ingestion. Similarly, an acute pulmonary edema in a subject free of heart disease should raise the suspicion of a toxic inhalational exposure. Acute cadmium fume poisoning should be suspected especially if soldering or welding had been performed, for iron and steel may be plated with cadmium. Metal poisoning should also be considered in the differential diagnosis of every case of oliguria or anuria of unknown etiology. A number of metals give rise to renal tubular dysfunction, in particular cadmium, uranium and lead. With lead the condition is seen more often in children. Lead poisoning should be considered in every case of glucosuria presenting in childhood. The causes of peripheral neuropathy are often obscure, and many cases remain incompletely diagnosed. Metal poisoning should be considered in the differential diagnosis of every case of peripheral neuropathy.

Few of the signs described in this chapter are specific to metal poisoning. A patient with a tremor does not necessarily have mercury poisoning, even if young and working with mercury. The tremor could be familial in origin, or could be due to other conditions. There are however certain specific signs which are indicative of metal absorption, though not necessarily of poisoning. Among these should be mentioned the characteristic garlic smell in the breath of selenium and tellurium workers, the green tongue of the vanadium worker and the pigmented alveolar margin, the lead line, due to the deposition of insoluble sulfide in the gums of lead workers. It should be mentioned that the lead line is not seen in edentulous workers, and that other metals will deposit sulfide in the same site. A finely mottled brown pigmentation of the skin, with leukodermia, a "rain drop pigmentation" affecting in particular the temples, eyelids and neck is seen in workers in contact with arsenical dusts. Ulceration and perforation of the nasal septum should also alert the clinician to previous long-term exposure to arsenical dusts or to chromates. Chronic ulceration on the fingers is seen following exposure to washing soda or to lime. White striae on the fingernails, Mees lines, are indicative of previous exposure to arsenic.

2.3 Toxicological analysis

A diagnosis of metal poisoning can be confirmed in the acute stage and often, but not always, in the chronic stage by finding an increased concentration of the suspected metal in the appropriate medium. Here it should be mentioned that the techniques involved in trace metal analysis in body fluids and tissues present certain difficulties and such analysis should only be performed in laboratories equipped for this purpose. Environmental sampling may need to be performed on air, water or food. Pills, residues in bottles and samples of vomit should be kept for chemical analysis after an acute poisoning incident. Blood, urine, feces and tissue samples may also require chemical analysis. The examination of blood and urine samples for metal concentration can be considered a routine procedure. However, it should be borne in mind that some metals disappear rapidly from the blood and others are sparingly excreted in the urine. In thallium poisoning, for example, the diagnosis can be confirmed by taking hair and nail clippings for analysis at a stage where blood and urine concentrations are not helpful. Evidence of methylmercury absorption can be obtained from analysis of mercury content of red blood cells and from hair samples, but methylmercury is only very slowly excreted in the urine, and analysis of urinary mercury is not helpful. In the diagnosis of lead poisoning difficulty may be caused where exposure has ceased some months previously. In such cases blood lead levels return to normal by the time diagnosis is attempted. A calcium EDTA provocation test will increase the urinary excretion of stored lead in such a situation.

2.4 Biochemical investigation

For most metal poisonings biochemical investigation is required in addition to estimation of metal concentrations in body fluids. The profile of biochemical abnormality is indicated in the chapters on the different metals considered. In general, biochemical tests of renal and of hepatic function are always required. Biochemical data are essential for diagnosis with certain toxic metals but are unhelpful with others. For example, in the diagnosis of lead poisoning it is essential to estimate δ-aminolevulinic acid and coproporphyrin in the urine as well as lead concentration in blood. In contrast, the diagnosis of poisoning by metallic mercury vapor or by alkylmercury compounds is essentially clinical together with estimation of metal concentrations in blood.

2.5 Physiological investigation

Electrocardiography, respiratory function tests, electroencephalography and nerve conduction studies provide examples of possible ancillary investigations for the diagnosis and for the determination of the extent of poisoning by certain metals. The principal disturbances of physiological function in such poisoning are indicated in the chapters on individual metals.

3. Treatment

The treatment of metal poisoning includes treatment of the acute and chronic effects, and suppression of tissue reaction where sensitization has occurred.

The management of acute metal poisoning requires emergency resuscitative procedures which may need to be initiated in the work or home environment and continued in an acute treatment unit. Those principles on which the management of the acute case of poisoning are based, which are aspects of emergency general medicine (Matthew and Lawson, 1975), will only be briefly referred to here, while therapy related to metal poisoning will be discussed in detail.

3.1 Prevention of further absorption

3.1.1 Removal from exposure

Absorption of a toxic metal may follow inhalation, ingestion or skin or mucosal contamination. Occupational exposure most frequently gives rise to absorption after inhalation. Following high-level inhalational exposure, the victim should be removed immediately from the contaminated atmosphere. Such an emergency situation may arise with volatile metal compounds such as stibine, arsine, alkylmercury, alkyltin and alkyllead compounds. Where removal from exposure cannot be carried out immediately, oxygen should be given. Following contamination of the skin with lipid-soluble metal compounds, contaminated clothing should be removed as soon as possible and the contaminated area irrigated and washed, but not rubbed, with copious cold water. Decontamination may also be required for hair and fingernails.

In chronic, cumulative metal poisoning, it may be sufficient to remove the subject from further exposure. In certain circumstances, for instance, a worker with mild poisoning following exposure to inorganic mercury vapor or to lead fume or dust, may require no therapy other than such removal. The normal excretory mechanisms will ensure a gradual recovery from mild toxic effects.

A review of possible routes of absorption in the individual case should include consideration of the possibility of exposure by contamination of food, drink, cigarettes or clothing. Where this possibility exists, food, drink and cigarettes at the work site should be prohibited, and facilities provided for showering and for a complete change of clothing after each shift.

3.1.2 Minimizing absorption from the gastrointestinal tract

3.1.2.1 Removal from the gastrointestinal tract

When the poison has been recently ingested some may be recovered by emptying the stomach. In a fully conscious patient this may be induced by vomiting. In an emergency situation, the simplest procedure is by stimulation of the back of the throat with a finger, or by giving a readily available emetic in the form of a spoonful of salt in a tumbler of water or syrup of ipecacuana, 30-45 ml for an adult. Where medical help is available, apomorphine 2-8 mg given intramuscularly produces rapid vomiting without untoward side effects.

The stomach may be washed out in a conscious patient following the passage of a gastric tube. If the cough reflex is absent, or consciousness is impaired, a cuffed endotracheal tube should first be passed to protect the lungs from inhalation of gastric contents. The procedure of gastric lavage is a skilled one which should only be performed in a properly equipped emergency department. It is probably worth attempting even if the poison had been swallowed several hours previously.

Following the passage of the toxic agent through the pylorus, absorption may be impeded by speeding its passage through the gastrointestinal tract. This can best be achieved by administering an osmotic purgative in the form of sodium sulfate, 10-30 g.

3.1.2.2 Inactivation in the gastrointestinal tract

While gastric lavage can be effectively performed with warm water, some poisons may be inactivated in the stomach by a specific antidote, and following lavage this may be left in the stomach in a volume not greater than 200-300 ml. However, lavage should not be delayed if the antidote is not immediately available. In an emergency situation egg white or milk will help to inactivate mercury and other heavy metals by precipitation in the stomach. Activated charcoal will effectively absorb poisons still present in the stomach (Holt and Holz, 1963). It should be given as a drink by mixing five to six five-ml spoonfuls of the powder in a glass of water, and this should be followed by aspiration 30 minutes later. Sodium bicarbonate solution will precipitate soluble iron salts in the stomach, or iron may be effectively chelated and thus inactivated with a solution of desferrioxamine of which 5-10 g in 100 ml water may be left in the stomach. Effective precipitation of the toxic barium ion can be produced by oral administration of gastric lavage with a 10% solution of sodium or magnesium sulfate. Following the ingestion of chromic acid, lavage should be performed with magnesium carbonate. Prussian blue will chelate thallium, and following its ingestion a colloidal solution should be given by duodenal tube at a rate of 250 mg per kg per day in divided doses.

3.2 General supportive therapy

In acute poisoning, resuscitative measures have to take precedence above all others. If the patient can be kept alive, excretory and detoxicating mechanisms together with the administration of specific antidotes, where these exist, will be able to ensure eventual elimination of the poison. Careful symptomatic medical care is always necessary, with an awareness of delayed effects which may occur.

3.2.1 Maintenance of respiration and circulation

Maintenance of respiration and circulation should receive precedence over all other procedures. The patency of the airway must be assured, especially at the site of the accident and on the journey to the treatment center, and artificial respiration and external cardiac massage may be necessary. At the treatment center tracheobronchial toilet may be required. The presence of hypoxia should be assessed by determining minute ventilation or preferably arterial oxygen and carbon dioxide tensions. Oxygen may be needed and also mechanical ventilation. Treatment for acute pulmonary edema may be necessary following exposure to beryllium or cadmium fume, nickel carbonyl, or to hydrogen selenide.

In an emergency situation a conscious shocked patient with a blood pressure below 80-90 mm Hg should be reassured, covered with blankets and kept supine with the legs elevated. The blood pressure may be raised as a temporary expedient, by giving a vaso-pressor drug, e.g. metaraminol, 2-5 mg given slowly intramuscularly or intravenously and repeated at intervals to keep the blood pressure about 100 mm Hg. Once the patient reaches the treatment center, the circulatory blood volume can be restored with suitable intravenous fluids. Blood transfusion may be required for severe hemolytic anemia following exposure to arsine.

3.2.2 Maintenance of water and electrolyte balance

Imbalance may occur from vomiting, diarrhea, tissue damage or from measures taken to eliminate the poison. It may be sufficient to give fluids by mouth to prevent dehydration, but intravenous infusion with appropriate biochemical monitoring is often required. An adequate urinary flow should be ensured and a catheter may be necessary.

Acute tubular necrosis may give rise to anuria following inhalational exposure to arsine or following the ingestion of mercuric or of uranium salts. Anuria has also followed poisoning with bismuth, copper and iron salts. Careful water and electrolyte balance has to be maintained until regeneration of the tubular epithelium leads to recovery.

3.2.3 Control of nervous system effects

The general supportive measures outlined above are usually adequate for the management of the patient whose level of consciousness is depressed. There is no indication for the use of analeptic drugs in acute metal poisoning. Convulsions, however, require management with a barbiturate or with diazepam 10-30 mg given parenterally. Cerebral edema may occur, as for example in acute lead poisoning. This should be treated with an osmotic agent, such as intravenous mannitol or a rapidly acting diuretic such as frusemide, together with a steroid in the form of hydrocortisone hemisuccinate or dexamethasone given intravenously. Prolonged sedation with barbiturates or diazepam may be necessary to overcome the hyperactivity and other behavioral disorders which may develop in poisoning with alkyllead compounds.

3.3 Elimination of absorbed poison

3.3.1 Diuresis

The promotion of a diuresis will increase the clearance of many poisons by decreasing their passive reabsorption from the proximal renal tubules. Diuresis can be achieved by giving a fluid load together with osmotic agents like mannitol or urea or by giving a pharmacological diuretic, e.g. frusemide. Furthermore, the excretion of some poisons is influenced by the pH of the urine, for passive tubular reabsorption is less effective with increased ionization of the solute in the tubular fluid. While of great value in some common forms of acute poisoning, these methods have some limitations in poisoning by metals, although in general, excretion of the toxic metal can be accelerated if a high urine flow is maintained, as for example in acute inorganic mercury and lead poisoning.

3.3.2 Biliary excretion

The action of certain toxic metals is prolonged as a result of an enterohepatic circulation where excretion in the bile can occur against a high concentration gradient followed by intestinal reabsorption. Osmotic purgatives may be of some value in decreasing transit time in the intestine and some of the poison may also be removed by means of duodenal intubation or by gall bladder drainage. A promising approach for interrupting this enterohepatic circulation has been described by Clarkson et al. (1973). This involves a complexing agent given by mouth which will bind with the metal compound excreted in the bile, prevent reabsorption, and enhance fecal excretion of those heavy metal compounds which undergo an extensive enterohepatic circulation. This synthetic polystyrene resin containing fixed sulfhydryl groups, when added to food in a concentration of 1%, doubled the rate of excretion of methylmercury from mice and lowered blood and tissue levels compared with untreated controls. In man mercury levels in blood were reduced and the fecal excretion of methylmercury enhanced (Bakir et al., 1973).

3.3.3 Dialysis

Extracorporeal hemodialysis will achieve far higher clearance rates of toxic metals not irreversibly bound to tissues than can be attained by forced diuresis. Metals known to be dialyzable are arsenic, copper, iron, lead, lithium, magnesium, mercury, potassium, sodium, strontium and zinc. The procedure is indicated where a potentally fatal dose has been absorbed, where the clinical condition is deteriorating in spite of adequate treatment by other means, and where a complication exists, such as aspiration pneumonia or renal insufficiency.

In several acute inorganic mercury poisoning, dialysis may be performed after giving dimercaprol to remove the mercury complex.

Cysteine, a sulfhydryl complexing agent, is capable of reversing the protein binding of methylmercury in the blood, and its infusion, together with hemodialysis, is effective in animals and may be effective in man in increasing the rate of elimination of methylmercury (Kostyniak et al., 1975).

3.3.4 Exchange transfusion

Where there are no facilities for hemodialysis or where the toxic material is poorly dialyzable, exchange transfusion may be life saving in severe poisoning by agents which remain in the blood in appreciable concentrations.

3.4 Inactivation of the absorbed poison

There are a limited number of therapeutic agents which may be administered to counteract the effects of an absorbed toxic metal. Such a specific antidote may act in different ways. It may combine with the toxic agent to form a less toxic or a non-toxic compound, which may be excreted more effectively in the urine; it may compete with the toxic agent and displace it from its receptor site; or it may displace the poison into a tissue where it cannot exert its toxic effects.

The intravenous administration of calcium gluconate will displace lead from its site of action and will temporarily relieve the intense pain of lead colic. An infusion of potassium has been shown to correct the potassium displacing capacity of absorbed soluble barium salts (Berning, 1975).

Certain antidotes have been designed specifically to compete for heavy metals with ligands essential for normal physiological function. These heavy metal antagonists, or chelating agents, form a stable complex with the metal in the form of a heterocyclic ring (Levine, 1975). The antagonists and the chelates produced are not themselves without toxic effects and they should not therefore be administered therapeutically in those mild cases of poisoning where removal from further exposure is sufficient to promote recovery.

3.4.1 Dimercaprol (BAL)

2,3 dimercaptopropanol (dimercaprol, British Anti-Lewisite, BAL) was synthesized as a specific antagonist to the vesicant arsenical war gases. It is a dithiol compound which successfully competes with protein sulfhydryl groups for arsenic compounds and for other heavy metals, by forming a stable chelate with them. The other metals for which dimercaprol has been shown to be effective are mercury in inorganic form, antimony, bismuth, cadmium, chromium, cobalt, gold, nickel. It is also used as an adjunct to edatate in the treatment of lead poisoning. However, to be effective it has to be given as early as possible in the course of poisoning. The half-life is short, metabolism and excretion being complete within four hours.

Dimercaprol is given by deep intramuscular injection as a 5 % solution in arachis oil (BP or a 10 % solution with benzyl benzoate in vegetable oil, USP). It is usually given in a dose of 3 mg per kg, 4 hourly, for the first two days, 6 hourly on the third day and thereafter once or twice daily for up to 7 days. In a severe acute case of poisoning 4-5 mg/kg should be given, 4 hourly for the first 24 hours, but no single dose should exceed 300 mg. There are a number of minor variations to this schedule. The drug should be administered at a lower dosage to patients with impaired renal function. It is wise to render the urine alkaline during therapy, as the dimercaprol-metal complex may dissociate in an acidic medium.

Dimercaprol has unpleasant and sometimes alarming side effects when given in full dosage. One of the most consistent is a rise in blood pressure accompanied by tachycardia. Other untoward effects are nausea and vomiting, headache, burning sensations in the mouth and throat, with paresthesiae of the hands, a feeling of constriction or of pain in the chest, lachrymation, salivation, rhinorrhea, sweating and abdominal pain accompanied by a feeling of anxiety.

Dimercaprol has been shown in the experimental animal to inhibit the blood pressure lowering effect of intravenously injected cadmium (Dalhamn and Friberg, 1954) and also of vanadium and of cobalt (Dalhamn et al., 1953; Dalhamn, 1953). While the urinary excretion of cadmium is enhanced by dimercaprol, Tepperman (1947) showed this to be accompanied by a large increase in cadmium concentration in the kidney and Gilman et al. (1946) observed severe renal damage as a sequel. While there may be an indication for giving dimercaprol in severe acute cadmium poisoning provided careful watch is kept on renal function, dimercaprol should never be given in chronic poisoning, where the cadmium concentration of the kidney is already high.

The administration of dimercaprol in mercury poisoning effects a redistribution of the body burden of the metal without increasing its excretion. In poisoning with inorganic mercuric salts dimercaprol decreases the renal concentration and thus protects the kidney. However, with mercuric salts, phenyl mercury compounds and also with methylmercury compounds, dimercaprol accelerates the uptake of mercury from blood into the tissues and in particular its uptake into the brain (Berlin and Rylander, 1964; Berlin and Lewander, 1965; Berlin et al., 1965). Dimercaprol is, therefore, contraindicated in the treatment of poisoning with both aryl- and alkylmercury compounds.

Dimercaprol enhances the toxicity of selenium and tellurium, producing kidney damage, and is contraindicated in poisoning by these metals (Amdur, 1958; Cewenka and Cooper, 1961).

3.4.2 Calcium disodium edatate (Calcium EDTA, Calcium versenate) Ethylene diamine tetraacetic acid and related compounds are able to chelate many divalent and trivalent metals in vitro. Infusion of the sodium salt will chelate calcium from the body and may result in hypocalcemic tetany. However, the calcium disodium salt, calcium disodium edatate (Calcium EDTA), is a valuable therapeutic agent for it will bind lead with the displacement of calcium from the chelate. However, it has dangerous toxic effects in the chelation of metals other than lead. Calcium EDTA is poorly absorbed from the gastrointestinal tract and so has to be given by intravenous infusion. It is distributed mainly in the extracellular fluid and is excreted rapidly by glomerular filtration, about 50 % appearing in the urine within one hour. Castellino and Aloj (1965) showed in rats a biphasic pattern of lead excretion, weakly bound extracellular lead being excreted rapidly, with lead complexed within cells being removed very slowly, probably as a direct result of an increased concentration gradient.

The toxic effects of calcium EDTA make it necessary to monitor its administration with care. The most important of these effects is on the kidney. Friberg (1956) showed that cadmium excretion could be increased considerably by giving Ca-EDTA to rabbits previously dosed with cadmium. However, severe degenerative and necrotic changes were seen in the tubular epithelium of rabbits with prolonged exposure to the metal. Reversible hydropic degeneration of the proximal tubules was produced in rats by Foreman et al. (1956) and proximal tubular damage has also been observed in man. It is not known whether this is due to a toxic effect of the chelating agent itself, to the chelated metal or to breakdown of the chelate in its transfer through the tubule, or even to the binding of a trace metal in an essential enzyme system. Untoward side effects in the treatment of lead poisoning include a febrile reaction with headache, myalgia, nausea and vomiting. Lachrymation, nasal congestion, mucocutaneous lesions, glucosuria, hypotension and ECG abnormalities have also been reported. Prolonged courses of calcium EDTA give rise to trace metal depletion, the most marked being the excretion of zinc.

Many of the side effects of calcium EDTA have been ascribed to excessive chelation following the administration of too high a dose over a short period of time. The drug is now usually administered in adults by the infusion of 1.0 g in 250 to 500 ml 5 % dextrose over a period of 1 to 2 hours. Two such infusions may be given daily for 3 to 5 days, but a daily dose of 50 mg per kg body weight should not be exceeded. Not more than 75 mg/kg should be given in 6 to 12 hours to a child. After an interval of a few days, during which time redistribution of lead from the intracellular to the extracellular compartments occurs, the course may be repeated. When the blood lead level is high, and especially where there is encephalopathy, Chisolm (1973) recommends combined BAL and calcium EDTA therapy. Renal function should be adequate before commencing treatment. While a patient is on calcium EDTA treatment, urinalysis should be performed and blood urea, electrolytes, calcium, phosphorus and alkaline phosphatase should be monitored regularly.

The local application of calcium EDTA in an ointment is of value in the treatment of chronic ulceration.

3.4.3 Penicillamine

β,β^1-dimethylcysteine or penicillamine, is derived from the hydrolytic degradation of penicillin. The D-isomer is an effective chelating agent for copper, iron, lead, mercury and zinc and it will increase the excretion of these metals in the urine. It is probably the chelating agent of choice in the less severe forms of lead poisoning. N-acetyl-dl-penicillamine may be even more effective than d-penicillamine in protecting against the effects of inorganic mercury (Aposhian and Aposhian, 1959), but there have been few valid comparisons. Penicillamine is well absorbed from the gastrointestinal tract and may thus be given by mouth. It is stable once absorbed and is excreted rapidly in the urine.

The most important side effects of penicillamine are acute sensitivity reactions manifested by fever, skin rashes, blood dyscrasias and occasionally renal tubular damage, proteinuria and the nephrotic syndrome. There is cross sensitivity with penicillin, so that persons who are allergic to penicillin should not be given penicillamine. Penicillamine antagonizes pyridoxine, although clinical effects following treatment with d-penicillamine are unusual.

Penicillamine is given orally, before meals, in four divided doses at a rate of 0.5 to 1.5 g daily, although up to 5 g daily has been given without ill effects. The urine should be tested for protein and a full blood count including a platelet count should be performed at weekly intervals, but where treatment continues for longer than 2 to 3 months, as in the management of Wilson's Disease, these tests may then be performed monthly.

3.4.4 Desferrioxamine mesylate

Desferrioxamine is a chelating agent with a remarkable affinity for iron but not for other metals. It is poorly absorbed from the gastrointestinal tract but following injection it complexes with iron and is rapidly excreted in the urine, increasing the excretion of iron. The iron in transferrin, the cytochromes and in hemoglobin is not accessible to desferrioxamine. Given by mouth it chelates iron remaining in the lumen of the gut and renders this unabsorbable and thus non-toxic. Its principal use is in the treatment of acute iron poisoning in children. It is also of value in increasing the excretion of iron in diseases associated with excessive iron storage, and in transfusion hemosiderosis.

The rapid intravenous administration of desferrioxamine may lead to an allergic or even anaphylactic reaction with hypotension and skin rashes. The drug should be used cautiously in patients with impaired renal function.

Desferrioxamine is given by slow intravenous infusion at a rate of not more than 15 mg per kg per hour to a maximum of 80 mg per kg per 24 hours. Desferrioxamine may also be given intramuscularly at a rate of 2 g for an adult, 1 g for a child, every 12 hours. Gastric lavage may be performed with 5 g desferrioxamine dissolved in 1 liter of water, and 5-10 g desferrioxamine in 50 to 100 ml water can be left in the stomach to chelate unabsorbed iron from the gastroinestinal tract.

3.4.5 Diethylenetriaminopentacetic acid (DTPA)

DTPA is a chelating agent with properties similar to those of Ca EDTA. It is the most effective agent known for the chelation of plutonium and its subsequent excretion in the urine, which may be increased by a factor of one hundred (Bair and Thompson, 1974). It may be given by slow intravenous infusion in a dose of 1 g on alternate days three times a week for three weeks.

3.4.6 Diethyldithiocarbamate (Dithiocarb)

Dithiocarb is a chelating agent which has been found to be of value in the treatment of acute nickel poisoning (Sunderman, 1971). Dithiocarb can be given orally in moderately severe poisoning, initially at a rate of 50 mg per kg in divided doses. In severe poisoning it has been given by Sunderman parenterally in an initial dosage of 25 mg per kg.

3.5 Modification of response

Under this heading are included examples of therapeutic measures in metal poisoning which are directed towards a modification of the tissue response to the poison, or to an alteration in the biochemical or metabolic state of the subject.

3.5.1 Modification of tissue response

Chronic beryllium disease is characterized by an inflammatory reaction which is granulamatous in nature and which appears to result from a hypersensitive response in certain individuals. The inflammatory process may be arrested, although not reversed, by adequate corticosteroid therapy. This treatment has resulted in a change in the clinical course of the disease with a reduction in symptoms and a favorable change in prognosis.

3.5.2 Modification of biochemical status

Chronic manganese poisoning has pathological, biochemical and clinical features which closely resemble those of Parkinson's disease, which once established, becomes a permanent disabling occupational disorder. The observations of Cotzias et al. (1971) on neurochemical similarities between the two conditions led to the administration of L-dopa in manganese-induced parkinsonism. Modification of the parkinsonian state in chronic manganese poisoning has been obtained with L-dopa (Mena et al., 1970). In the majority of patients so treated, hypokinesia and rigidity were markedly reduced and postural reflexes improved with a restitution of balance. There was no evidence of an increased body burden of manganese in the parkinsonian ex-miners treated in this way. Beneficial effects can be considered solely as a form of replacement therapy.

REFERENCES

Alexander, C.S. (1972). Amer. J. Med. 53, 395-417.

Amdur, M.L. (1958). AMA Arch. Ind. Health 17, 665.

Aposhian, H.V. and Aposhian, M.M. (1959). J. Pharmacol. Exp. Therap. 126, 131-135.

Bair, W.J. and Thompson, R.C. (1974). Science 183, 715-722.

Bakir, F., Damluji, S.F., Amin-Zaki, L., Murtadha, M., Khalid, A., Al-Rawi, N.Y., Tukriti, S., Dhahir, H.I., Clarkson, T.W., Smith, J.C. and Doherty, R.A. (1973). Science 181, 230-241.

Beattie, A.D., Moore, M.R. and Goldberg, A. (1972). Lancet 2, 12-15.

Beritic, T. (1971). Arch. Environ. Health 23, 289-291.

Beton, D.C., Andrews, G.S., Davies, H.S., Howells, L. and Smith, G.F. (1966). Brit. J. Ind. Med. 23, 292.

Berlin, M. and Rylander, R. (1964). J. Pharmacol. Exp. Therap. 146, 236-240.

Berlin, M. and Lewander, T. (1965). Acta Pharmacol. Toxicol 22, 1-7.

Berlin, M., Jerksell, L.G. and Nordberg, G. (1965). Acta Pharmacol. Toxicol. 23, 312-320.

Berning, J. (1975). Lancet 1, 110 (letter).

Castellino, H. and Aloj, S. (1965). Brit. J. Ind. Med. 22, 172-180.

Cavanagh, J.B., Fuller, N.H., Johnson, H.R.M. and Rudge, P. (1974). Quart. J. Med. 43, 170, 293-319.

Cerwenka, E.A. and Cooper, W.C. (1961). Arch. Environ. Health 3, 189-200.

Chisolm, J.J. (1973). New Engl. J. Med. 289, 1016-1018.

Clarkson, T.W., Small, H. and Norseth, T. (1973). Arch. Environ. Health 26, 173-176.

Cotzias, C.G., Papavasiliou, P.S., Ginos, J., Steck, A. and Diiby, S. (1971). Ann. Rev. Med. 22, 305-326.

Dalhamn, T. (1953). Acta Pharmacol. Toxicol. 9, 259.

Dalhamn, T. and Friberg, L. (1954). Acta Pharmacol. Toxicol. 10, 199-203.

Dalhamn, T., Forssman, S. and Sjöberg, S.G. (1953). Acta Pharmacol. Toxicol. 9, 11.

Foreman, H., Finnegan, C. and Lushbaugh, C.C. (1956). J. Amer. Med. Ass. 160, 1042-1046.

Friberg, L. (1956). AMA Arch. Ind. Health 13, 18-23.

Friberg, L., Piscator, M., Nordberg, G. and Kjellström, T. (1974). "Cadmium in the Environment." CRC Press, Cleveland.

Gilman, A.F., Philips, S., Allen, P. and Koelle, E. (1946). J. Pharmacol. 87, 85.

Goyer, R.A. and Rhyne, B.C. (1973). Int. Rev. Exp. Pathol. 12, 1-77.

Holt, L.E. and Holz, P.H. (1963). In: "Activated Charcoal as an Antidote for Poisons." pp 1-3. National Clearinghouse Poison Control Center, USA.

Hunter, D. (1975). "The Diseases of Occupations". (5th edition) English Universities Press, London.

Jenkins, G.C., Ind, J.E., Kazantzis, G. and Owen, R. (1965). Brit. Med. J. 2, 78-80.

Kazantzis, G. (1971). Int. J. Environ. Studies 1, 301-306.

Kazantzis, G. (1975). 18th Int. Congr. Occup. Health, Abstracts, p. 285.

Kostyniak, P.J., Clarkson, T.W., Cestero, R.V., Freeman, R.B. and Abbasi, A.H. (1975). J. Pharmacol. Exp. Therap. 192, 260-269.

Landrigan, P.J., Whitworth, R.H., Baloh, R.W., Staehling, N.W., Barthel, W.F. and Rosenblum, B.F. (1975). Lancet 1, 708-712.

Levine, W.G. (1975). In: "The Pharmacological Basis of Therapeutics." (L.S. Goodman and A. Gilman, eds) pp 912-923. (5th edition) Macmillan, New York.

Matthew, H. and Lawson, A.A.H. (1975). "Treatment of Common Acute Poisonings." (3rd edition) Churchill Livingstone, Edinburgh.

Mena, I., Court, J., Fuenzalida, S., Papavasiliou, P.S. and Cotzias, G.C. (1970). N. Engl. J. Med. 282, 3-8.

Nordberg, G.F. (1976). "Effects and Dose-Response Relationships of Toxic Metals." Elsevier, Amsterdam.

Stoeckle, J.D., Hardy, H.C. and Weber, A.L. (1969). Amer. J. Med. 46, 545-561.

Sunderman, F.W. (1971). Ann. Clin. Res. 3, 182-185.

Teng, C.T. and Brennan, J.C. (1959). Radiology 73, 354-361.

Tepperman, H.M. (1947). J. Pharmacol. 89, 343.

MUTAGENIC AND CARCINOGENIC EFFECTS OF METALS

George Kazantzis and Lorna Lilly

1. Mutagenic effects

1.1 Introduction

Genetic damage can be caused by naturally occurring or industrially produced chemicals, including metallic compounds, in the human environment. This may have two important consequences. The first of these is that the damage done to the genes of the exposed population in the present generation may be manifested as an increase in abnormal individuals in later generations. The second important consequence of genetic damage is indicated by the growing body of evidence which correlates the ability of an agent to cause genetic damage with its carcinogenicity. We do not wish to defend the hypothesis that only genetic damage can result in cancer, but rather to point out the astonishingly high correlation between agents which are able to induce mutation and those which are able to induce cancer. This is reflected in the current attempts to use systems involving genetic damage for short-term carcinogenicity testing (see e.g. WHO, 1974; Ames et al., 1975; Bridges, 1976).

Genetic damage may be defined as damage to deoxyribonucleic acid (DNA). It is usual to classify such damage under three main headings:

1) Changes at the level of the nucleotide bases in the DNA ("point mutations")
2) Gross chromosomal aberrations
3) Changes in chromosome number

1.2 Types of genetic damage

1.2.1 Point mutations

Mutations may be thought of as a change in the nucleotide bases of the gene such that replicated copies of that gene give rise to abnormal daughter cells, which if they are gametes, will pass the abnormality on to the next generation. Such a change would occur spontaneously only with a very low frequency. However, in the presence of a mutagen the induced mutation rate may be many times higher than the spontaneous rate.

Mutations which involve base substitution such as one purine for another purine, or a pyrimidine for another pyrimidine in the DNA are known as transitions, while mutations which involve the substitution of a purine for a pyrimidine or a pyrimidine for a purine are known as transversions. Those mutations which involve the deletion or addition of bases are called frameshift mutations.

The ability to induce point mutations may be most conveniently and extensively tested on microorganisms. However, higher animals (including mammals) may be used. Although some mutagens may be species specific (for example those that require the presence of a particular activating enzyme) others are more generally mutagenic. Since the ultimate target of a mutagen is DNA, the demonstration of positive results in any one organism should alert us to the possibility that it may be mutagenic in man.

The "point" mutations which are of most concern to man are the recessives which may remain hidden for many generations after their induction, until a chance marriage between two carriers (heterozygotes) produces an offspring carrying a double dose of the defective gene (homozygote) who will then be in some way abnormal. Typical of the many human conditions produced by recessive genes are the numerous inborn errors of metabolism such as phenylketonuria, sex-linked recessive conditions such as hemophilia, and cystic fibrosis which is probably the commonest of all inherited diseases among Caucasians, occurring with a frequency of 1 in every 2,000 births.

1.2.2 Gross chromosomal aberrations

Chromosomal aberrations involve gross damage to the chromosomes such as to be readily visible under the light microscope. Such damage will include chromosome or chromatid breaks resulting in the loss of fragments of chromosome containing various amounts of genetic information. More striking abnormalities may be induced by the joining up of broken ends of two or more chromosomes or chromatids to form a single configuration (e.g. exchanges, translocations, rings etc). Such abnormalities may be unstable and later form anaphase bridges which interfere with the mechanical separation of the daughter nuclei and which may cause cell death. Acentric fragments which are unable to attach to the mitotic or meiotic spindle will be lost from the daughter nuclei and may also cause cell death. Many early miscarriages in humans may be due to such unstable aberrations. In animal experiments, inherited lethality occurring in the first generation of offspring (dominant lethality) is usually due to chromosomal aberrations. However, chromosomal aberrations need not be lethal, and can be passed on to later generations. Such aberrations may be genetically unbalanced. For example the translocation form of Down's syndrome (mongolism) results in 1/3 of live births being affected in each generation.

Since chromosomal aberrations are only scorable during cell division, the tissues used for this purpose must either be dividing *in vivo* (e.g. bone marrow, or the root tips of plants) or must be capable of being cultured *in vitro* (e.g. human lymphocytes).

1.2.3 Changes in chromosome number

Some environmental agents may cause an increase or decrease in the number of chromosomes present in a cell (aneuploidy). Such changes may be due to the chromosomes failing to separate regularly at cell division (non disjunction). The faulty distribution of chromosomes may be caused by various events such as an abnormality in the mitotic spindle, along which chromosomes travel to the daughter cells, or by a deletion of the centromeres by which the chromosomes are attached to the spindle.

Agents which destroy the spindle, resulting in a failure to distribute chromosomes normally to daughter cells, are said to cause "C-mitosis" (or "C-meiosis") and the consequences of their action can be observed visually under the microscope. Changes in chromosome number can give rise to a wide range of inherited abnormality, including for example the trisomy form of Down's syndrome (mongolism) and the syndromes of sex chromosome anomaly.

In summary, the three kinds of damage described above could lead to one of literally hundreds of inherited clinical disorders. It should be emphasized that no single test will detect all kinds of genetic damage, that not all mutagens are able to cause all types of damage, and that different organisms may respond differently to any particular mutagen.

Although most of the papers published on the genetic effects of environmental agents concern the types of damage described above, the effects on other genetic processes such as mitotic and meiotic recombination, gene conversion etc, are occasionally described. Lack of space will not permit us to discuss these or other interesting subjects such as the mutations found in some organisms, which confer resistance to metals (Tuovinen and Kelly, 1974), the role of metal ions on the modification of the mutagenicity of non-metallic mutagens (Shamberger et al., 1973), or the role of metals in the production of mutagenic mycotoxins in stored foodstuffs (Gupta and Venkitasubramanian, 1975).

1.3 Principal metals showing mutagenic effects

A number of metals have been shown to induce mutagenic effects in biological material ranging from plant cells and viruses to animal and human cells including those exposed *in vivo*. As a result, the possible role of metals as a genetic hazard to human health is being increasingly recognized. With most metals the capacity to cause genetic damage to human germ cells cannot yet be evaluated owing to the inadequate nature of the data available, but there seems no reason to suppose that these cells are necessarily less sensitive than other tissues of the body, or that they differ markedly in this respect from the germ cells of other mammals. Some of the more important data on the genetic effects of metals are summarized below.

1.3.1 Mercury (alkyl, aryl and inorganic compounds)

Ceresan-M (containing ethyl (n-phenyl-p-toluene-sulphonanilide) mercury) was believed to have been responsible for two earlier outbreaks of mercury poisoning in Iraq in 1956 and 1960 (Mathews and Al-doori, 1976). These authors tested Ceresan-M for mutagenicity. They fed 30 and 40 mg of the fungicide per 100 mg of food to the larvae of the fruit fly *Drosophila melanogaster*. This resulted in a significant increase in the frequency of sex-linked lethal mutations.

Other fungicides such as Granosan (containing ethylmercuric chloride) and Agrimix M (containing ethylmercuric chloride and phenylmercuric-dinaphthyl methanedisulfonate) are known to affect the mitotic spindle, causing "C-mitosis" (Fishbein, 1974).

Ramel (1972a) in his extensive review on the genetic effects of mercury compounds drew attention to their interference with the mitotic spindle leading to an abnormal distribution of chromosomes and resulting in aneuploidy. In experiments upon *Allium cepa* Ramel compared the "C-mitosis" inducing ability of methyl- and phenylmercury hydroxide and other compounds with the similar action of colchicine. From this and the works of other authors which he reviewed, Ramel established two very important points. The first of these is that organic mercurials are even stronger spindle inhibitors than colchicine, some compounds such as butylmercury bromide and methylmercury hydroxide being effective at a concentration as low as 0.1×10^{-6}M and 2.5×10^{-7}M respectively, compared to the 200×10^{-6}M threshold activity of colchicine. (The effects of methylmercury on human cells are of the same order of magnitude as upon *Allium cepa*.) The second important point made by Ramel is that whereas low concentrations of colchicine tend to produce a complete blockage of the spindle fiber mechanisms, the mercurials produce a gradual transition from normal spindle to complete C-mitosis, passing through a state where spindle damage is slight, leading to the defective distribution of single chromosomes. Both of these observations are of the greatest practical importance, the former because low levels of environmental mercury contamination occur widely, and the latter because the loss and gain of single human chromosomes are the basic cause of human abnormalities such as Turner's, Kleinfelter's and Down's syndromes. Defects caused by a complete C-mitosis, which concerns the whole chromosome set, are likely to be lethal at an early stage in development and therefore less important.

Ramel has carried out experiments the results of which support the hypothesis that organic mercury acts upon the spindle fibers by interacting with sulfhydryl groups of the protein units involved in spindle fiber formation.

The effects of mercury are not limited to mitosis. Ramel mentions his own unpublished experiments which show that methylmercury can induce "C-meiosis" in the reproductive tissues of flower buds. He also reports experiments with Drosophila melanogaster in which he showed that the effects of methylmercury on the non disjunction of chromosomes can be detected as genetic abnormalities in the offspring of these fruit flies. Ramel also obtained chromosome breakage with both phenyl- and methylmercury in the root tips of Allium cepa.

From his study of the effects of methylmercury on the repair of radiation-induced chromosome breaks, Ramel assumes that the chromosomal breakage by methylmercury is due to its action directly on the chromosomes rather than on enzyme systems of chromosome repair and DNA synthesis. Umeda et al. (1969) have reported an increase of abnormal mitotic cells in treatment with inorganic, phenyl- and alkylmercuric compounds on HeLa cells. They found "severance of the chromosomes to form rod shaped masses", polynuclear cells and an increase of the mitotic index.

Kato (1976) demonstrated that methylmercuric chloride caused an increase in chromosomal aberrations in human lymphocytes after in vitro and in vivo exposure.

The effects of methylmercury on human chromosomes have been studied by Skerfving et al. (1974). They examined lymphocytes from the cultured blood of 23 subjects exposed to methylmercury as a result of a diet of contaminated fish. These were compared with the lymphocytes of 16 unexposed controls. There were statistically significant relationships between frequencies of cells with chromatid type aberrations, "unstable" chromosome type aberrations, and aneuploidy, and the blood cell mercury levels in the exposed people. No correlations were found between stable type chromosome aberrations and blood mercury levels.

1.3.2 Lead

Organic lead compounds are more effective spindle inhibitors than inorganic forms. In experiments with Allium cepa root tips Ramel (1972b) demonstrated that the active concentration of organic compounds of lead, mercury, and tin able to cause spindle damage was very low, i.e. 10^{-6} or 10^{-7}M, while with inorganic metals the doses required are 100-1000 times greater. One of the interesting possibilities which arises from Ramel's work is that the effects of different metals may be additive and therefore the total metal load in the environment may be important.

Although the ability of lead to cause spindle damage (and therefore euploidy) seems well established, there is some conflicting evidence as to whether it also causes chromosome aberrations.

Levan (1945) showed that triethyl lead at a concentration of 10^{-5}M produced chromosome bridges and fragments in Allium cepa but that trimethyl lead did not produce a corresponding effect. No evidence of an increased yield of aberrations was found by Bauchinger et al. (1972) in a chromosomal analysis of the lymphocytes of 29 policemen with increased blood levels of lead. Bauchinger and Schmid (1972) failed to find an increase in aberrations in cell cultures of the Chinese hamster after treatment with various concentrations of lead acetate. Schmid et al. (1972) examined 32 workers in the lead manufacturing industry, three with acute lead intoxication, and found no evidence of increased aberration yield compared with a group of twenty persons from a normal population. They also found no evidence of increased aberrations in human lymphocytes treated with lead acetate *in vitro*.

However, an increase in chromosomal aberrations in occupationally exposed people has been reported by Schwanitz et al. (1970) and Forni and Secchi (1972). The latter authors found a significant increase in aberrations in 15 workers with preclinical intoxication and in 37 workers with clinical poisoning, but not in workers with a history of lead poisoning who had been unexposed during the previous 18 months. Increased yields in aberrations have also been found in human lymphocytes treated *in vitro* with lead acetate (Schwanitz et al., 1970; Beek and Obe, 1974; Obe and Beek et al., 1975). Deknudt et al. (1973) classified the workers which they observed into three groups, (1) people exposed to high levels of zinc and low levels of lead and cadmium, (2) people exposed to high levels of all three metals, (3) people exposed to lead and cadmium in the absence of zinc. They concluded that exposure to cadmium and zinc did not appear to increase the number of cells with severe chromosome abnormalities and that lead was responsible for the chromosome and chromatid aberrations seen. Hickey et al. (1974) have applied regression techniques to data collected from 38 metropolitan regions which showed that the concentration of atmospheric lead was a significant predictor of infant mortality rate and congenital malformations.

The mutagenic effect of lead on algae *(Platymonas subcordiformis)* was investigated by Hessler (1975), who found no increased incidence of mutation in experimental conditions where nitrosoguanidine (an inducer of point mutations) and U.V. radiation induced a high proportion of mutants.

1.3.3 Cadmium

There is some evidence that cadmium is able to induce genetic damage. Oehlkers (1952) cites the unpublished experiments of Gläss in which roots of *Vivia faba* were exposed to cadmium nitrate. The optimum concentration at which cadmium nitrate induces chromosomal breakage is about M/10,000 which makes it about 200 times more active than the chromosome breaker urethane.

Röhr and Bauchinger (1976) treated cell cultures from Chinese hamsters with various concentrations and various lengths of exposure to cadmium sulfate. Treatments with 10^{-5} to 10^{-4} mol/l for 3 hours caused "C-mitosis" and structural chromosome aberrations mainly of the chromatid type. After 16 hours of treatment with 10^{-4} to 10^{-8} mol/l, marked chromosome stickiness and picnosis appeared at the higher concentration.

Cadmium is known to kill spermatozoa and cause testicular interstitial cell tumors in mammals. Gilliavod and Leonard (1975) investigated the genetic effects of cadmium in mice. A single intraperitoneal injection of 1.75 mg/kg cadmium chloride did not increase the dominant lethals in male mice during the first three weeks after treatment and failed to induce translocations in the F_1 male offspring. The treated males were killed three months later and their testes examined for translocation configurations. None were found.

Chromatid breaks and exchanges and acentric fragments have been found in the peripheral lymphocytes in 24 workers in a zinc melting plant who had increased blood levels of lead and cadmium (Bauchinger et al., 1976). Because of their earlier negative findings on lead the authors attribute these aberrations to either cadmium or the combined effects of lead and cadmium.

Shiraishi (1975) examined the chromosomes of 12 Japanese patients with Itai-Itai disease. Eight of these patients had a high number of chromatid aberrations and the other four had a significant number of stable chromosome type aberrations. The frequency of aneuploidy in the cells of all 12 was higher than in cells from controls. Shiraishi also cites earlier work in which she had shown similar damage in cultured human lymphocytes treated *in vitro*.

Bui et al. (1975) in a chromosomal aberration analysis of five cadmium exposed Swedish workers, four Japanese Itai-Itai patients and seven Swedish and Japanese controls found no evidence that cadmium induces chromosome damage *in vivo* in man. The Itai-Itai patients as well as the Japanese control subjects demonstrate a significantly higher frequency of chromosomally abnormal cells than the cadmium-exposed Swedish workers and the controls.

The conflict between the findings of Shiraishi and those of Bui outlined above makes the interpretation of the effects of cadmium difficult. However, the facts that Gläss established that cadmium was capable of causing chromosome breakage in *Vicia*, and that both Shiraishi and Röhr and Bauchinger obtained effects with mammalian cells treated *in vitro* (human and hamster respectively), do suggest that cadmium is capable of producing chromosome aberrations under some conditions. Certainly, in the absence of further evidence, the possibility that it may also do so *in vivo* in man should not be overlooked.

Cadmium chloride injected *in vivo* has been shown to cause changes in chromosome numbers in metaphase II oocytes of mice. These changes were of such types as would have caused monosomy, trisomy or triploidy in subsequent progeny (Shimada et al., 1975, 1976). Similar results were obtained with hamster oocytes (Watanabe et al., 1976).

1.3.4 Chromium

Venitt and Levy (1974) have demonstrated that simple hexavalent chromium salts such as Na_2CaO_4, K_2CaO_4 and $CaCrO_4$ are able to induce point mutations in "spot tests" in which the chemical to be tested is dropped onto plates prespread with the bacterium

E. coli. Three strains were used, two of which lacked certain DNA repair enzymes. The positive results from these experiments were confirmed with others in which bacteria in suspension were treated with Na_2CrO_2. Soluble salts of other metals such as tungsten and molybdenum were also tested as was a soluble trivalent chromium compound $Cr_2SO_4K_2SO_4 \cdot 2H_2O$. These were found not to be able to induce mutation. Similar tests with salts of other metals such as zinc, cadmium and mercury were also negative. The absence of either the exrA ("error prone-repair") or the uvrA ("excision repair") pathway did not modify the mutagenic response to chromate. Venitt et al. conclude that chromates fall into the transition-causing class of mutagens. It is highly likely that they specifically attack guanine-cytosine base pairs in the DNA, causing guanine-cytosine → adenine-thymine transitions in the subsequent round of DNA replication.

1.3.5 Selenium

Five selenium compounds were tested for their effects on human lymphocyte chromosomes by Nakamuro et al. (1976). Chromosomal aberrations, mainly of the chromatid type, were found to increase in frequency with increasing dose. Most were gaps, but there was a substantial increase in breaks and exchanges as well. The order of efficiency of the compounds used was H_2SeO_3, Na_2SeO_3, SeO_2, H_2SeO_4, Na_2SeO_4. Tests with strains of B. subtilis having or lacking recombination capability were carried out. These experiments suggested that damage to DNA was produced by selenites but not by selenates. Experiments to evaluate the effects of selenium components on the loss of transforming activity of B. subtilis DNA showed that treatments with H_2SeO_3 and SeO_2 yield a 30% loss of transforming activity compared to untreated DNA, but no significant effects were obtained using Na_2SeO_4, H_2SeO_4 or Na_2SeO_3.

1.3.6 Manganese

Manganese is reported to be able to induce "petite" mutations in resting and growing cells of yeast (Prazmo et al., 1975). These mutations involve changes in mitochondrial DNA rather than in nuclear DNA. Putrament et al. (1975) demonstrated the induction of antibiotic resistant mutants in yeast mitochondrial DNA by manganese. From these results the authors suggested that manganese acts as an error producing factor on replicating mitochondrial DNA. The authors think the most likely hypothesis is that the cation acts directly with mitochondrial DNA polymerase.

Dube and Loeb (1975) have shown that manganese acts as a mutagenic agent during *in vitro* DNA synthesis and Hall and Lehman (1968) have shown that manganese reduces the fidelity of DNA synthesis *in vitro*.

In 1965 Orgel and Orgel showed that manganese was mutagenic in bacteriophage T4. The manganous ion induces mutations that are reversible by 5 Bu or 2 amino-purine and which are therefore presumably of the transition type.

1.3.7 *Zinc*

Kroeger (1964) reported an effect of zinc chloride on the puffing of the larval chromosomes of *Chironomus thummi*. These effects are similar to those produced by the mutagen urethane and by ecdysone. Carpenter and Ray (1969) found no significant effect of zinc chloride on the production of dominant lethals or sex linked recessive mutation in Drosophilia. However, there was an effect with radioactive $^{65}ZnCl_2$.

Herich (1969) showed that zinc sulfate inhibited the passage of cells from interphase to prophase and caused a disturbance in the differentiation of the chromosome matrix and precocious dispiralization of the chromosomes. The effect of zinc ions on mitosis was also studied by Barlow (1966), using cells of wing anlagen of *Ephestia kuhniella z. in vitro*. The numbers of nuclei in prophase rose steeply to a peak after three hours of treatment in 0.02M $ZnCl_2$. Treatments of more than two hours significantly raised the number of metaphases. Barlow concluded that zinc blocks metaphase by disrupting the mitotic spindle.

Organic zinc compounds may be used as fungicides. Pilinskaya (1970) reported that chromosomal aberrations were present in the blood of 9 industrial workers who had worked in a ziram plant for 3-5 years. An increased incidence of both chromosome and chromatid type aberrations was found in their lymphocytes.

Blood from unexposed persons (Pilinskaya, 1971) treated *in vitro* with ziram also contained chromosomal aberrations. In a survey of patients treated with a closely related organic compound disulfiram (Antabuse) containing no zinc, and with ethyl instead of methyl groups, Lilly (1975) was unable to find a significant increase in aberrations. It is possible that the difference between the action of ziram and disulfiram may be due to the presence and absence of zinc molecule respectively.

1.3.8 Nickel

Cultured rat embryo muscle cells exposed to nickel showed a marked decrease in mitotic index and in induced abnormal mitotic figures such as multipolar spindles, C-metaphase-like shapes, disoriented bipolar spindles, lagging chromosomes and unequal chromosome divisions (Swierenga and Basrur, 1968). Histochemical staining for protein-bound sulfhydryl groups showed that nickel could be causing mitotic abnormalities by interfering in the sulfhydryl groups involved in the spindle mechanism.

Levan (1945) pointed out unusual results with roots of *Allium cepa* exposed to nickel nitrate. The chromosomes behave as in "C-mitosis", but nevertheless the spindle remains clearly visible.

Nickel chloride, which does not give tumors in animals, also gave negative results in two mutagenicity tests with *Salmonella typhimurium* in host-mediated assay (Buselmaier et al., 1972).

Corbett et al. (1970) showed that nickel sulfate at 300 μg/ml is toxic but not mutagenic to intracellular bacteriophage T4.

1.3.9 Arsenic

Arsenical interference with normal DNA repair processes in E. coli has been reported by Rossman et al. (1975).

Petres et al. (1970) examined the lymphocytic chromosomes of 13 people who had been exposed to arsenic and also the chromosomes of unexposed controls. They found a large increase in acentric fragments, chromatid bridges and dicentric chromosomes in the exposed group, and they point out that this is in agreement with their earlier in vitro work. Petres and Hagedorn (1977) found a similar increase in patients treated with arsenicals.

Elevated sister chromatid exchange rates were found by Burgdorf et al. (1977) in the lymphocytes of six individuals who had taken arsenic and later developed stigmata of arsenic use, including multiple cutaneous malignancies. Sister chromatid exchanges are a type of aberration which can only be observed after differential staining of the chromatids. Their biological importance is not fully understood, but they may be induced by many chromosome-breaking substances.

Oppenheim and Fishbein (1965) were able to induce chromosome aberrations in cultured human lymphocytes by treatment with potassium arsenite.

1.3.10 Other metals

Levan (1945) has shown that "colchicine mitosis" (C-mitosis) is caused by large numbers of inorganic metals salts such as those of lithium, beryllium, chromium, iron, cobalt, nickel, copper, arsenic, rubidium, palladium, cadmium, barium, gold, mercury, thallium, lead. With many of these metals the threshold of "C-mitosis" was considerably lower than that for colchicine. For example the threshold for lead nitrate and copper nitrate was as low as 0.000005-0.00005 M. There was a tendency for the threshold to fall with increasing molecular weight. Levan also showed that pronounced chromosome stickiness was caused by titanium, lithium, beryllium, aluminum, iron, cobalt, nickel, bismuth, molybdenum, and thallium.

Radiation can cause genetic damage, so radioactive isotopes of metals such as uranium can be mutagenic in this way. However, it has been pointed out (United Nations, 1972) that high concentrations of uranium isotopes may present a toxicological rather than a radiological hazard. We do not know of any reports of genetic experiments specifically designed to distinguish between the radiation and chemical effects of uranium. However, Malenkho and Bakun (1973) have reported aneuploidy in human lymphocytes and mouse muscle cells after treatments with up to 0.5 μg/ml and 0.3 μg/ml respectively of uranium nitrate. Chromosome stickiness has also been reported by Levan.

Cobalt is known to cause "petite" (mitochondrial) mutations in yeast (Prazmo et al., 1975).

Oehlkers (1952) referred to his earlier work on meiotic chromosomes and to the work of Deufel (1951) with mitotic chromosomes of *Vicia faba* both of which had shown that aluminum chloride was a potent chromosome breaker.

Many chemicals used for anti-tumor therapy are able to induce mutation or chromosome breaks. One such agent is the platinum(II) coordination complex cis-$PtCl_2(NH_3)_2$ which causes DNA cross links. Monti-Bragadin et al. (1975) showed that $PtCl_2(NH_3)_2$ causes mutation in *Salmonella typhimurium*. The strain of *Salmonella* used is specifically reverted by base-pair substitution mutagens.

Sub-toxic doses of antimony sodium tartrate and ammonium tellurate have been reported to induce chromosome aberrations *in vitro* in cultured human lymphocytes (Paton and Allison, 1972).

2. Carcinogenic effects

2.1 Definition, mechanism of action

A carcinogen is a substance exposure to which results in the eventual appearance of a cancer which would not have occurred otherwise. A chemical carcinogen may initiate malignant change by direct action or by a variety of indirect mechanisms. The only common mechanisms of action known to exist between widely varying carcinogenic chemicals relate to their electrophilic reaction, which makes them capable of reacting with various nucleophilic centers in the cell such as nucleic acids and proteins, to their ability to be converted by metabolic processes to electrophilic reagents or to their properties as free radicals. The mechanism of action of the carcinogenic metals is far from clear at present. They appear to be direct acting or ultimate carcinogens, although their oxidation state may influence their carcinogenic potential (Mancuso, 1975) and salts of the same oxidation state may have different carcinogenic effects (Sunderman, 1973).

2.2 Recognition and evaluation of carcinogenic activity

2.2.1 Clinical observation

That some metals may act as carcinogens was first realized from clinical observation of industrial workers. The carcinogenic potential of nickel was recognized some 30 years after the opening of the Mond Nickel Refinery in South Wales, when 10 cases of nasal cancer, a rare tumor in the general population, were reported. Observations by astute clinicians of an association between cancer and an environmental or behavioral factor have on several occasions been the starting point of detailed epidemiological enquiry. Such observations are likely to be as valuable in the future as they have been in the past.

2.2.2 Epidemiological enquiry

The carcinogenic potential of chemicals can only be investigated in humans by means of epidemiological study. Such study is capable of identifying high risk groups together with their antecedent host and environmental factors. However, interactions between environment and host factors, and even between environmental exposures, are complex and require sophisticated methods for their investigation (Fraumeni, 1975). With regard to the metals, exposure in relation to occupation is likely to have been greater than general environmental exposure and the study of occupational groups is thus of particular value. However, exposure patterns in industry up to the present time have only been available retrospectively, rendering them but approximations. Furthermore, the exposure patterns involving the suspected carcinogenic metals are difficult to characterize and to record. Epidemiological study can tell us that a number of industrial processes involving exposure to certain metals carry an increased risk of cancer, but it may only be possible to define this risk in terms of an occupation, an industrial plant or a process (Norseth, 1976). Apart from the problems involved in complex exposures over long periods of time, further difficulties in evaluating the carcinogenic effect of one specific metal are caused by host and behavioral variables which may increase or decrease the carcinogenic response.

Smoking histories have been deficient in past epidemiological studies, and the carcinogenic effect of tobacco smoke could not be assessed. Dietary pattern, exposure to other chemicals or drugs, hormonal and immune responses may also provide important modifications. The multifactoral nature of the risks, then, mean that sophisticated methods are required for the evaluation of the carcinogenic potential of an individual metal. At the present time, the only metals which have been shown convincingly by epidemiological study to cause human cancer are arsenic, chromium and nickel, while beryllium and cadmium are highly suspected.

2.2.3 Bioassay

Many metals have been tested for carcinogenic activity in experimental animals but the animal models used have often borne no relation to the human exposure situation in terms of dose or route of administration. Since human exposure occurs only by inhalation or ingestion it is difficult to interpret the significance of cancer induced in the rat by a large dose of an insoluble compound of the metal given by intramuscular or subcutaneous injection. For example, cadmium sulfide suspensions will produce sarcoma in a high proportion of rats injected in this way (Kazantzis, 1966) but there is no evidence at present that the inhalation of insoluble cadmium sulfide dust can give rise to cancer in man. Nevertheless, in our present state of knowledge, it is probably wise to work on the assumption that certain individuals would be at risk of developing cancer if exposed to a chemical carcinogen definitely active in one or more animal models. Basic biochemical systems in man and animals are sufficiently similar to make it likely that man would not react differently from animal models except as regards quantitative aspects and tissues affected. Conversely most chemical agents shown by epidemiological study to give rise to cancer in humans have also demonstrated carcinogenic activity in one or more animal models (Weisburger and Rall, 1972). At an international symposium at the Karolinska Institute in 1977 (Task Group on Air Pollution and Cancer, 1978) on risk assessment methodology and epidemiological evidence of cancer, it was concluded that if a substance has been shown to be carcinogenic in an adequate animal test system, it should normally be dealt with as if it is also carcinogenic in man, unless adequate epidemiologocal evidence exists to the contrary. In borderline cases, the report concludes, error should be on the side of safety.

To give reliable information, test systems using animal models have to be very carefully designed and controlled. Various factors have to be taken into account in the selection of animal species, strains, sex and age. Because of the possibility of interactions and of biologic effects the purity of the metal compound to be tested must be assured. Careful thought should be given to the correct use of control animals, the route of administration and the evaluation of results, taking into account the experimental design. The correct use of animal models is further considered by Weisburger (1976).

2.2.4 In vitro test systems

Much of the information we have at present on the carcinogenic effects of metals has been obtained by bioassay using animal models. However, the technique is always time-consuming and expensive and it may even be misleading, especially when applied to the detection of weak carcinogens. The need to obtain a rapid, if only provisional, answer to the question as to whether a chemical in current or proposed use has a carcinogenic potential has led to intense activity in an attempt to devise an appropriate indicator system which *in vitro* will mimic molecular receptors (Stoltz et al., 1975). Such tests, briefly indicated below and reviewed by Weisburger (1976), should at present be regarded as not more than screening procedures.

2.2.4.1 Mutagenicity tests

It has been found empirically that many chemical carcinogens in their reactive form are also powerful mutagens in microbial and mammalian test systems. However, certain carcinogens have not been shown to exert mutagenic activity and some powerful mutagens do not appear to be carcinogenic. The congruence between mutagenic and carcinogenic effects became evident with the development of techniques for converting a precarcinogen into the ultimate carcinogen, and a premutagen to the ultimate mutagen (Ames, 1971; Ames et al., 1973). The present state of knowledge on the value of mutagenicity tests in evaluating the carcinogenic effect of chemicals can be summed up as follows (see WHO, 1974; Lancet, 1977).

While the relationship between carcinogenesis and mutagenesis requires further investigation, the association between mutagenicity and carcinogenicity of many compounds is sufficiently great to justify the use of mutagenicity tests in prescreening for possible carcinogenesis. The results of mutagen testing, to be evaluated, will require correlating with figures for the incidence of cancer in populations known to be exposed to particular hazards.

2.2.4.2 Cell culture transformation

Cell cultures can be transformed *in vitro* by the addition of a carcinogen and these will give rise to abnormal cell lines when reimplanted into their host (Williams et al., 1973). This technique may not allow for adequate biochemical activation, but a further development, in which the carcinogen is administered to a pregnant animal, and tissue cultures prepared from the fetus, will do so (DiPaolo and Nelson, 1973).

2.2.4.3 DNA damage and repair

There are differences in the type of DNA damage which can be induced by carcinogenic and non-carcinogenic chemicals. Study of these breaks and their repair processes may be of value in the identification of carcinogenic activity (Stich and San, 1973).

2.2.4.4 Serum protein indicators

Carcinogens which act primarily on the liver have been shown to induce serum α-feto protein in the rat long before the appearance of the tumor (Kroes et al., 1973). This observation is being developed as a screening test for the carcinogenic potential of a limited group of chemicals.

2.3 Principal metals showing carcinogenic effects

Only three metals have been shown in epidemiological studies to exert an unequivocal carcinogenic effect in humans. These metals are nickel, chromium and arsenic. However, adequate epidemiological studies and in particular prospective studies, have not been performed and human data are at present greatly lacking. There is some evidence, at present inconclusive, that cadmium may exert a carcinogenic effect and possibly also beryllium. Most of the available epidemiological data have been obtained retrospectively on occupationally exposed groups. Of the three metals known to have exerted a carcinogenic effect in humans, only nickel and chromium have been confirmed as carcinogens in experimental animals, there being no conclusive evidence in the case of arsenic. Cadmium and beryllium, which are suspect in humans, have been shown to have an unequivocal carcinogenic effect in animals, although the exposure situations have not been comparable. In addition to the above, iron, cobalt, zinc, titanium and lead have produced tumors in experimental animals but the doses used and modes of administration have been entirely foreign to any conceivable human exposure situation. These ten metals will be considered in greater detail below.

2.3.1 Nickel

2.3.1.1 Epidemiological observations

A highly significant excess of both lung cancer and cancer of the nasal sinuses has been found in nickel refinery workers in different countries (Mastromatteo, 1967; Doll et al., 1970; Virtue, 1972; Pedersen et al., 1973; Saknyn and Shabynina, 1973; Sunderman and Mastromatteo, 1975). In the Mond Nickel Works in South Wales, where exposure to nickel carbonyl gas had occurred in the past, Doll et al. (1970) estimated the excess mortality from lung cancer to be ten times that expected for men first employed before 1914 and about five times that expected for those first employed between 1915 and 1924. For nasal cancer, those first employed between 1910 and 1914 experienced an almost 900-fold increase, while those first employed between 1920 and 1924 had a mortality almost 100 times the national average. Recent observations by Doll (1975) showed that the risk to the refinery workers continued at least until 1929. While all the men studied were manual workers in the refinery, only some of these were employed on the specific process. Others were general laborers who changed their job frequently. It was thus not possible to collect detailed information on exposure.

The theory that nickel carbonyl had acted as a specific carcinogen had to be abandoned when a similar hazard was found in nickel refinery workers in Ontario, where only the electrolytic process had been used (Mastromatteo, 1967). Sunderman (1973) collected data on 327 cases of lung cancer and 115 cases of cancer of the nasal sinuses in nickel refinery workers with a variety of exposure to nickel in Norway, France, Germany, USA and Japan as well as in Wales and Canada.

Apart from nickel refinery workers, isolated cases of lung and nasal cancer have been reported in workers in nickel plating, nickel grinding and nickel polishing operations but the significance of these cases cannot be assessed as no definitive epidemiological study has yet been performed. However, from the extent of exposure in the old nickel refining processes and from the fall in cancer mortality when this was reduced, it seems unlikely that the much lower levels of exposure encountered in nickel plating would constitute a risk.

The exact identity of the carcinogenic agent or agents in nickel refineries is open to speculation. Sunderman and Mastromatteo (1975) consider that fresh nickel dusts from sintering and roasting processes are especially carcinogenic, but also speculate on respirable particles of metallic nickel subsulfide and nickel oxide as significant carcinogens. The available data, which are inadequate, suggest that the cancer risk is greater in heavy cigarette smokers with an interaction between two carcinogens as in the case of asbestos and smoking.

The latent period from first exposure to nickel to diagnosis of cancer is long, averaging about 25 years with a range of some 10 to 40 years. In those cases for which information is available, epithelial tumors predominated but anaplastic and pleomorphic tumors have also been observed (Sunderman, 1973).

2.3.1.2 Animal models

The experimental models which have been used to study nickel carcinogenesis are reviewed by Sunderman (1973). Carcinogenesis has been adequately demonstrated in several animal species following inhalational and parenteral administration of nickel but not following oral or cutaneous exposure. Nickel sulfide, Ni_3S_2, is the most potent metallic compound yet tested with regard to carcinogenesis, subcutaneous or intramuscular injection, having given rise to fibrosarcoma or rhabdomyosarcoma (Heath and Daniel, 1964; Sunderman, 1976). (The high yield of tumors and the ease with which they can be produced and propagated makes this method a conveniently simple one for experimental tumor induction.) Metallic nickel powder in suspension and in the form of pellets or sponge has produced similar tumors following implantation. Lung cancer has been produced in guinea pigs following the inhalation of nickel dust (Hueper, 1958) and in rats following the inhalation of nickel carbonyl (Sunderman and Donnelly, 1965). Carcinoma of the kidney and cholangiosarcoma of the liver in addition to sarcoma have been observed following multiple intravenous injections of nickel carbonyl (Lau et al., 1972).

While comparison of the relative potency of the various nickel compounds tested is difficult because of variation in experimental design from study to study, potency appears to be inversely related to solubility of the nickel compound in aqueous media, the highly soluble salts like nickel chloride having no apparent carcinogenic activity. Various possible mechanisms of nickel carcinogenesis have been proposed. Beach and Sunderman (1970) have observed an inhibition of RNA synthesis in hepatocytes, believed to be an inhibitory effect of nickel on RNA polymerase activity, thus altering the expression of genetic information.

2.3.2 Chromium

2.3.2.1 Epidemiological observations

A lung cancer hazard in workers producing chromates from the raw material, chromite or chrome iron ore was first noted in Germany in the 1930's. The observation was confirmed in the USA by Machle and Gregorius (1948) and in Britain by Bidstrup and Case (1956). Similar observations have been made in the 1970's in the Soviet Union, Japan and Norway (Pokrovskaya and Shabynina, 1973; Ohsaki et al., 1974; Langård and Norseth, 1975). In the American series the observed mortality for lung cancer was about 30 times the number expected from the national mortality data of that time, but in the British series the increased risk was computed at three times that expected.

In the industrial process the raw chromite is dried, crushed, mixed with calcium and sodium or potassium carbonate and roasted in a rotary furnace in an oxidizing atmosphere. The insoluble oxide is converted to a water soluble monochromate and the dichromate produced by treating this with dilute sulfuric acid. As the lung cancer hazard has only been demonstrated convincingly in workers producing chromates and not in workers exposed to dichromate or chromic acid, it seems reasonable to suppose that the carcinogenic agent is present in the primary ore or in one of the furnace products either as an intermediate or as a component of the residue (Kazantzis, 1972). However, Pokrovskaya (1973) reported an increased incidence of lung cancer in chrome alloy production and Mancuso (1975) considers all forms of chromium to be carcinogenic. There are no studies available today which unequivocally show that chromium compounds other than hexavalent ones are carcinogenic (Langård and Norseth, 1977).

The time interval from the first exposure to chromium compounds to the findings of lung cancer has varied from 10 to 47 years, with a mean latent interval from 10.6 to 21 years in different studies. About 80% of the lung cancers examined were squamous cell carcinomas, but undifferentiated cancers and adenocarcinomas have also been seen.

2.3.2.2 Animal models

Animal models have not contributed so far to the precise identification of the carcinogen in the chromate production industry although they confirm the carcinogenic potential of the metal. A small number of tumors have been observed in the rat following the intramuscular or intrapleural administration of chromite ore roast (Hueper, 1958), and more tumors followed the intramuscular implantation of calcium chromate (Hueper and Payne, 1959). A small number of rats developed lung cancer (6 squamous, 2 adenocarcinoma in 100 rats) following bronchial implantation of calcium chromate in the form of a pellet (Laskin, 1968). Inhalation exposure to calcium chromate dust produced bronchial adenocarcinoma in mice in a single experiment (Nettesheim et al., 1971). Levy and Venitt (1975) produced squamous cell carcinoma of the rat lung by implanting medium solubility hexavalent chromium salts into the bronchial tree. In their animals without lung tumors, a significant increase in squamous metaplasia was seen in those rats exposed to the pure hexavalent compounds but not in those exposed to others. These authors also tested a number of hexavalent and trivalent chromium compounds for mutagenic activity. Again they observed significant levels of induced mutation with hexavalent chromium compounds, but none with soluble trivalent compounds.

2.3.3 Arsenic

2.3.3.1 Epidemiological observations

The evidence for the carcinogenicity of arsenic is based on observations made in occupational groups and also in general population groups exposed to arsenic following ingestion in food, water or medicaments or following inhalation in arsenic polluted atmospheres.

A number of different occupational groups with exposure to arsenic have shown an excess cancer risk. An increase in observed lung cancer deaths over those expected was found in workers engaged in the production of sodium arsenate (Hill and Faning, 1948), in refinery and smelter workers (Lee and Fraumeni, 1969; Milham and Strong, 1974; Tokudome and Kuratsune, 1976; Pinto, 1977), and in miners (Osburn, 1969). Arsenic is a component of ores containing copper, lead, zinc, silver and gold, nickel or cobalt and is also associated with natural radioactive sources as in the Erzgebirge. Exposure patterns are therefore complex and often involve other suspected carcinogens. Although exposure may occur to a variety of arsenic compounds, refinery workers and others are predominantly exposed to airborne arsenic trioxide. German and French vineyard workers who used a pesticide spray containing arsenic developed chromic arsenic poisoning over a 12- to 14-

year exposure period. Roth (1958) investigated 27 deaths and found 12 cases of lung cancer, one of which was bilateral, among these workers. These deaths occurred 8 to 14 years after the pesticides had been banned.

Epidemiological observations have been made in retrospective and in correlation studies, and the relative risk of developing respiratory cancer has been computed at 3.3, based on 147 observed and 44 expected deaths (IARC, 1973).

Skin cancer following chronic arsenic poisoning and following medication with arsenical preparations, in particular with Fowler's solution, has been consistently reported (Neubauer, 1947). The characteristic pathogenesis consists of keratotic lesions persisting for many years, distributed particularly on palms and soles, and eventually progressing to epithelioma, which may be multifocal in origin. Chronic lesions resembling eczema or psoriasis which progress to a low grade basal cell carcinoma may also occur (Bowen's disease). Bowen's disease may be associated with an increased incidence of other malignant lesions, but this has not been confirmed. There have been a number of case reports of multicentric arsenic-induced epithelioma associated with lung cancer, sometimes bilateral in origin. Cancers of the lymphatic and hemopoietic systems, and hemangiosarcoma of the liver, the latter associated with hepatic cirrhosis and splenomegaly (Banti's disease), have also been reported to occur more frequently following arsenic exposure, both with and without epithelioma (Lander et al., 1975; Popper and Thomas, 1975). However, the excess risk has not yet been adequately confirmed epidemiologically.

The daily intake of arsenic in food and water is of the order of 100 to 800µg, with an average value of 190µg/day (Schroeder and Balassa, 1966; Somers and Smith, 1971). It is usually present in its pentavalent form, although traces of trivalent arsenic derived from pesticides may also be found (Grasso and O'Hare, 1976). Contamination of food and drinking water from industrial sources and subsequent observations of chronic arsenic poisoning have been widely reported (see Tsuchiya et al., 1977). In one such episode in Taiwan (Yeh and How, 1963; Tseng, 1977) various clinical manifestations, including hyperkeratotic skin lesions were seen in the exposed population. Arsenic levels in drinking water were of the order of 0.8 to 2.5 mg/l. The rate of skin cancer was very high, 10.6/1000 population, and evidence of a dose-response relationship was found.

Arsenic is one of the large number of carcinogens identified in tobacco smoke, but its significance remains to be determined. Raised levels of arsenic have been detected in the atmosphere and in soil in the vicinity of non-ferrous smelters and refineries, and raised levels of urinary arsenic in the neighborhood population (Milham and Strong, 1974). In a large scale epidemiological study in the USA average mortality rates from lung cancer were significantly increased in countries with copper, lead or zinc smelting or refining industries compared with countries where other non-ferrous ores were processed (Blot and Fraumeni, 1975). After taking confounding variables into account, the authors concluded that the excess mortality could well have been caused by industrial emissions containing inorganic arsenic.

As with nickel and chromium, the latent interval between first exposure to arsenic and development of cancer is long. Average latent intervals of 34, 39 and 41 years were estimated for heavy, medium and light exposure categories (Lee and Fraumeni, 1969).

Further references to skin and lung cancer following arsenic exposure are given by Hernberg (1966) and by Tsuchiya et al. (1977).

2.3.3.2 Animal models

Surprisingly, there is little if any convincing evidence for the carcinogenic effect of arsenic in experimental animals. An increased incidence of lymphocytic leukemia and of lymphoma was observed in female Swiss mice and their young following the subcutaneous injection of sodium arsenate during pregnancy (Osswald and Goettler, 1971). However, repeated application of an aqueous solution of sodium arsenate failed to elicit any skin tumors (Baroni et al., 1963). Many other negative findings have been reported.

2.3.4 Cadmium

2.3.4.1 Epidemiological observations

In contrast to arsenic, there is little epidemiological evidence at present to support the contention that cadmium is a cancer hazard for man, while animal models show cadmium to have a considerable carcinogenic potential.

Three out of eight deaths in 74 men with at least 10 years of exposure to cadmium oxide dust were noted by Potts (1965) to be from cancer of the prostate. Kipling and Waterhouse (1967) surveyed 248 workers exposed to cadmium oxide dust for a minimum period of one year. They found four cases of prostatic cancer, where the expected figure was computed at 0.58, from annual incidence rates supplied by the Birmingham Regional Cancer Registry. They concluded that the increased incidence of prostatic cancer was highly significant (probability of occurrence 0.003), while the incidence of other forms of cancer was close to that expected. However, the numbers involved were not large (they included the 3 cases noted by Potts) and the authors were unable to infer the existence of an industrial hazard. Since then, only one study has supported the above findings, a retrospective cohort mortality investigation of workers exposed to cadmium fume and cadmium oxide and sulfide dusts (Lemen et al., 1976). Atmospheric samples taken in the premould department showed concentrations of 74.8 and 90.3 $\mu g/m^3$ for cadmium and 0.3 and 1.1 $\mu g/m^3$ for arsenic respectively. Samples in the retort department showed 11.05 $\mu g/m^3$ for cadmium and 1.4 $\mu g/m^3$ for arsenic. The study involved 292 workers with a minimum of 2 years of exposure during a 30-year period from 1940. The group experienced a total of 92 known deaths (99.3 expected). A significantly excessive risk was demonstrated for malignant neoplasms, with 27 observed and 17.6 expected ($p<0.05$). Twelve of these deaths were due to respiratory cancer (5.11 expected, $p<0.05$) and 4 were due to prostatic cancer (1.15 expected, N.S.). The greatest risk for respiratory cancer was apparent 30 years after initial employment and for prostatic cancer 20 years after initial exposure. At that time interval, the increased risk was significant as the expected figure was only 0.88 ($p<0.05$). However, in this study there was concomitant exposure to arsenic, and smoking histories have not yet been made available.

2.3.4.2 Animal models

Finely divided cadmium metal suspended in fowl serum injected into the thigh muscle of the rat gave rise to rhabdomyosarcoma and fibrosarcoma (Heath and Daniel, 1964). Similar tumors followed the intramuscular and subcutaneous injection of cadmium sulfide and of cadmium oxide suspensions (Kazantzis, 1963; Kazantzis and Hanbury, 1966). The tumors were metastasizing and pleomorphic and the tumor yield was high. In the series with cadmium oxide, the tumors were comparable with the nickel sulfide-induced tumors described earlier. Subcutaneous and intramuscular injection of soluble cadmium chloride or sulfate, in addition to producing sarcomata at the injection site, also induced interstitial cell tumors of the testis (Haddow et al., 1964; Roe et al., 1964; Reddy et al., 1973), even after a single dose (Gunn et al., 1963). The interpretation of the specific role of cadmium in initiating interstitial cell tumors of the testis is difficult. The initial effect of cadmium is a necrotizing one on the interstitial cells, probably acting through the vascular supply to the testis, and it is possible that the neoplastic change is secondary to this. Furthermore, certain strains of laboratory rats have since been shown to have a high spontaneous incidence of interstitial cell tumors (Levy et al., 1973).

In an experimental series in which cadmium sulfate was given by subcutaneous injection or by gastric instillation repeatedly over a prolonged time interval to rats and to mice, in the latter by gastric instillation only, no morphological change attributable to cadmium was seen in the prostate gland or in other parts of the genitourinary system. The dose range was based on exposure levels calculated to produce low molecular weight proteinuria in man. The rats were given weekly subcutaneous injections of 0.05, 0.1 or 0.2 mg 3 $CdSO_4 \cdot 8H_2O$ weekly gastric instillation of 0.2, 0.4 or 0.8 mg/kg for 2 years and the mice were given weekly gastric instillations up to 4 mg/kg for 18 months, equivalent to 1.75 mg Cd/kg body weight (Levy et al., 1973; Levy and Clark, 1975; Levy et al., 1975). This dose range was considered to be too low for adequate carcinogenic evaluation (IARC, 1973).

2.3.5 Beryllium

2.3.5.1 Epidemiological observations

There is very little epidemiological evidence to support the contention that exposure to beryllium compounds provides a cancer risk in man. Mancuso (1970) examined the records of the Beryllium Case Registry (Hardy et al., 1967) and, from a total of 800 cases, found no overall evidence of an increased mortality from lung cancer, compared with a control cohort. There did appear to be a small increase in lung cancer mortality in workers with a short exposure and in particular in those with a previous history of respiratory disease. Bayliss (1972), who investigated the causes of death among 3900 men who had worked in American beryllium factories, found no increase in mortality from respiratory cancer.

2.3.5.2 Animal models

The carcinogenic activity of beryllium compounds, in particular of the oxide, sulfate, phosphate and zinc beryllium silicate, has been confirmed by a number of investigations in rabbits, rats, mice and monkeys. Intravenously administered beryllium oxide and zinc beryllium silicate have given rise to osteogenic sarcoma in mice and rabbits (Gardner and Heslington, 1946; Barnes et al., 1950). Inhalational exposure has also been followed by osteosarcoma (Dutra et al., 1951). In other series where beryllium oxide, sulfate or phosphate was administered by inhalation to rats and monkeys, lung cancer was produced (Vorwald and Reeves, 1959; Schepers, 1964; Reeves et al., 1967). The cell types included adenocarcinoma as well as squamous cell carcinoma.

Taking the various experimental observations together, a dose-response relationship for the production of malignant tumors in animal models becomes apparent, and the quantitative aspects of this are given by Reeves (1977).

Beryllium has been shown to block thymidine incorporation into hepatic DNA (Witschi, 1970) and also certain enzymes needed for DNA synthesis. It has been suggested that beryllium binding to nucleoproteins leads to specific inhibition of events leading to DNA replication (Reeves, 1977).

2.3.6 Iron

2.3.6.1 Epidemiological observations

Speculation has been put forth on the role of iron oxide as a possible carcinogen common to hematite, asbestos, nickel and chrome workers but very little evidence exists in support of such a hypothesis. Boyd et al. (1970) observed 36 deaths from lung cancer in hematite miners in Cumberland, England, compared with 21 expected, based on regional mortality figures, this being a significant difference. An increased lung cancer mortality has also been noted in the iron miners of Kiruna, Sweden (Jörgensen, 1973). However, in both occupational environments there are also increased concentrations of radon daughters. Cigarette smoking and diesel fumes have also been queried as confounding variables.

2.3.6.2 Animal models

Iron dextran and a few other polysaccharide complexes have induced local sarcomas in mice, rats, hamsters and rabbits following subcutaneous or intramuscular injection in high dosage (e.g. Richmond, 1959; Haddow and Horning, 1960; Roe and Carter, 1967). An increased incidence of non-lymphoreticular neoplasms at sites distant to the injection has also been observed in mice (Langvad, 1968) but this observation requires confirmation. The significance of these observations in relation to iron therapy in man has been debated. The possible hazard is reviewed by Roe (1967). While parenteral iron polysaccharide therapy is common, sarcoma at the injection site has been rarely reported.

2.3.7 Lead

2.3.7.1 Epidemiological observations

Mortality studies of lead workers have not shown any evidence of an excess risk for malignant neoplasms (Dingwall-Fordyce and Lane, 1963; Robinson, 1974).

2.3.7.2 Animal models

Numerous investigators have confirmed the observation initially made by Zollinger (1953) on the induction of renal carcinoma and adenoma in rats given lead phosphate by subcutaneous injection. Similar results have been obtained with lead acetate given subcutaneously or by mouth to rats and also to mice (Van Esch and Kroes, 1969; Coogan, 1973). Interstitial cell tumors of the testis have followed prolonged feeding with lead acetate in rats (Zawirska and Medras, 1968). The doses used in all these experiments were high and the significance with regard to human environmental exposure, while unknown, is unlikely to be great.

2.3.8 Zinc

2.3.8.1 Epidemiological observations

There are no epidemiological observations to suggest that zinc may present an increased cancer hazard to man.

2.3.8.2 Animal models

The production of teratomas by the injection of zinc chloride into the testes of chickens was the first experimental observation to be made on the carcinogenic action of metal compounds (Sunderman, 1976). Since then the observation has been amply confirmed by several investigators. The subject is reviewed by Sunderman (1976) who could find no evidence that zinc compounds are carcinogenic after administration by any route other than intratesticular injection.

2.3.9 Cobalt

2.3.9.1 Epidemiological observations

There is no evidence to suggest that cobalt may present an increased cancer hazard to man.

2.3.9.2 Animal models

Metallic cobalt powder, cobalt oxide and cobalt sulfide have been shown to induce local sarcomas following subcutaneous intramuscular or intraosseous injection (Vollman, 1940; Heath, 1956; Gilman, 1962). Injection into muscle gave rise to rhabdomyosarcoma, as seen following the administration of nickel and of cadmium. Heath et al. (1971) were able to produce such neoplasms with fine particles from prostheses made from a cobalt chromium alloy.

2.3.10 Titanium

2.3.10.1 Epidemiological observations

There is no evidence to suggest that titanium may present an increased cancer hazard to man.

2.3.10.2 Animal models

An increased incidence of fibrosarcoma and of lymphosarcoma has been observed in rats given titanium metal powder in long-term survival studies (Furst, 1971). Tumors were also obtained in rats and mice with titanocene injected intramuscularly. Fibrosarcoma was induced at the injection site and hepatomas and malignant lymphomas were also obtained (Furst and Haro, 1969).

REFERENCES

Ames, B.N. (1971). In: "Chemical Mutagens: Principles and Methods for Their Detection." (A. Hollander, ed) p 267, Vol. 2, New York.

Ames, B.N., Dunston, W.E., Yamasaki, E. and Lee, F.D. (1973). Proc. Natl. Acad. Sci. 70, 2281.

Ames, B.N., McCann, E., Choi, E. and Yamasaki, E. (1975). Proc. Natl. Acad. Sci. 72, 5135-5139.

Barlow, C.A. (1966). Nature 209, 1045-1046.

Barnes, J.M., Denz, F.A. and Sissons, H.A. (1950). Brit. J. Cancer 4, 212-222.

Baroni, C., van Esch, G.J. and Saffiotti, U. (1963). Arch. Environ. Health 7, 668-674.

Bauchinger, M. and Schmid, E. (1972). Mutat. Res. 14, 95-100.

Bauchinger, M., Schmid, E. and Schmid, T. (1972). Mutat. Res. 16, 407-412.

Bauchinger, M., Schmid, E., Einbrodt, H.J. and Dresp, J. (1976). Mutat. Res. 40, 57-62.

Bayliss, D. (1972). In: "NIOSH Criteria for a Recommended Standard: Occupational Exposure to Beryllium." US Department of Health, Education, and Welfare, Washington, D.C.

Beach, D.J. and Sunderman, F.W. (1970). Cancer Res. 30, 1645-1650.

Beek, B. and Obe, G. (1974). Experientia 30, 1006-1007.

Bidstrup, P.L. and Case, R.A.M. (1956). Brit. J. Ind. Med. 13, 260-264.

Blot, W.J. and Fraumeni, J.F. (1975). Lancet 2, 142-144.

Boyd, J.T., Doll, R., Faulds, J.S. and Leiper, J. (1970). Brit. J. Ind. Med. 27, 97-105.

Bridges, B. (1976). Nature 261, 195-200.

Bui, T., Lindsten, J., Nordberg, G.F. (1975). Environ. Res. 9, 187-195.

Burgdorf, W., Kurvink, K. and Cervenka, J. (1977) Hum. Genet. 36, 69-72.

Buselmaier, von W., Röhrborn, G. and Propping, P. (1972). Biol. Zentralbl. 91, 311-325.

Carpenter, J.M. and Ray, J.H. (1969). Amer. Zool. 9, 1121.

Coogan, P.S. (1973). Proc. Inst. Med. Chicago 29, 509.

Corbett, T.H., Heidelberger, C. and Dove, W.F. (1970). Mol. Pharmacol. 6, 667-679.

Deknudt, Gh., Leonard, A. and Ivanov. B. (1973). Environ. Physiol. Biochem. 3, 132-138.

Deufel, J. (1951). Chromosoma 4, 239-272.

Dingwall-Fordyce, I. and Lane, R.E. (1963). Brit. J. Ind. Med. 20, 313-315.

DiPaolo, J.A. and Nelson, R.L. (1973). Arch. Pathol. 95, 380.

Doll, R. (1975). Personal communication.

Doll, R., Morgan, L.G. and Speizer, F.E. (1970). Brit. J. Cancer 24, 623-632.

Dube, D.K. and Loeb, L.A. (1975). Biochem. Biophys. Res. Commun. 67, 1041-1046.

Dutra, F.R., Largent, E.J. and Roth, J.L. (1951). AMA Arch. Pathol. 51, 473-479.

Fishbein, L. (1974). Sci. Total Environ. 2, 341-371.

Forni, A. and Secchi, G.C. (1972). In: "Environmental Health Aspects of Lead." pp 473-485. Published by the Commission of European Communities Directorate General for Dissemination of Knowledge, Center for Information and Documentation CID, Luxembourg.

Fraumeni, J.F. (1975). In: "Persons at High Risk of Cancer. An Approach to Cancer Aetiology and Control." (J.F. Fraumeni, ed) Academic Press, New York.

Furst, A. (1971) Geol. Soc. Amer. Mem. 123, 109-130.

Furst, A. and Haro, R.T. (1969). Prog. Exp. Tumour Res. 12, 102-133.

Gardner, L.U. and Heslington, H.F. (1946). Fed. Proc. 5, 221.

Gilliavod, N. and Leonard, A. (1975). Toxicology 5, 43-47.

Gilman, J.P.W. (1962). Cancer Res. 22, 158-162.

Grasso, P. and O'Hare, C. (1976). In: "Chemical Carcinogens." (C.E. Searle, ed) p 721. American Chemical Society, Washington D.C.

Gunn, S.A., Gould, T.C. and Anderson, W.A.D. (1963). J. Natl. Cancer Inst. 31, 745-759.

Gupta, S.K. and Venkitasubramanian, T.A. (1975). Appl. Microbiol. 29, 834-836.

Haddow, A. and Horning, E.S. (1960). J. Natl. Cancer Inst. 24, 139-147.

Haddow, A., Roe, F.J.C., Dukes, C.E. and Mitchley, B.C.V. (1964). Brit. J. Cancer 18, 667-673.

Hall, Z.W. and Lehman, I.R. (1968). J. Mol. Biol. 36, 321-333.

Hardy, H.C., Rabe, E.W. and Lurch, S. (1967). J. Occup. Med. 9, 271-276.

Heath, J.C. (1956). Brit. J. Cancer 10, 668-673.

Heath, J.C. and Daniel, M.R. (1964). Brit. J. Cancer 18, 124.

Heath, J.C. and Daniel, M.R. (1964). Brit. J. Cancer 18, 251-264.

Heath, J.C., Freeman, M.A.R. and Swanson, S.A.V. (1971). Lancet 1, 564-566.

Herich, R. (1969). Nucleus 12, 81-85.

Hernberg, S. (1976). Paper presented to Symposium on Origins of Human Cancer, Cold Spring Harbour Laboratory, New York.

Hessler, A. (1975). Mutat. Res. 31, 43-47.

Hickey, R.J., Clelland, R.C., Boyce, D.E. and Bowers, E.J. (1974). Mutat. Res. 26, 445-446.

Hill, A.B. and Faning, E.L. (1948). Brit. J. Ind. Med. 5, 2-6.

Hueper, W.C. (1958). AMA Arch. Ind. Health 18, 284-291.

Hueper, W.C. (1958). Arch. Pathol. 65, 600-607.

Hueper, W.C. and Payne, W.W. (1959). Amer. Ind. Hyg. Ass. J. 20, 274.

IARC (1973). "Evaluation of Carcinogenic Risk of Chemicals to Man." Vol. 2, pp 1-181. International Agency for Research on Cancer, WHO, Geneva.

IARC Working Group on the Evaluation of the Carcinogenic Risk of Chemicals in Man (1973). IARC Monograph, Vol. 2, Lyon.

Jörgensen, H.S. (1973). Work Environ. Health 10, 107-125.

Kato, R. (1976). Jap. J. Hum. Genet. 20, 256-257.

Kazantzis, G. (1963). Nature 198, 1213.

Kazantzis, G. (1966). Brit. J. Cancer 20, 190.

Kazantzis, G. (1972). Ann. Occup. Hyg. 15, 25-29.

Kazantzis, G. and Hanbury, W.J. (1966). Brit. J. Cancer 20, 190-199.

Kipling, M.D. and Waterhouse, J.A.H. (1967). Lancet 1, 730-731.

Kroeger, H. (1964). Chromosoma 15, 36-70.

Kroes, R., Williams, G.M. and Weisburger, J.H. (1973). Cancer Res. 33, 613.

Lancet (1977). 2, 340-341.

Lander, J.J., Stanley, R.J., Sumner, H.W., Boswell, D.C. and Aach, R.D. (1968). Gastroenterology 68, 1582-1586.

Langvad, E. (1968). Brit. J. Cancer 3, 415-423.

Langård, S. and Norseth, T. (1975). Brit. J. Ind. Med. 32, 62-65.

Langård, S. and Norseth, T. (1977). In: "Toxicology of Metals." Volume II, pp 164-187. Environmental Health Research Series, 600/1-77-022, Environmental Protection Agency, Research Triangle Park.

Laskin, S. (1968). American Industrial Health Association, St. Louis. Quoted by Roe, F.J.C. and Carter, R.L. (1969). Brit. J. Cancer 23, 172.

Lau, T.C., Hackett, R.L. and Sunderman, F.W. (1972). Cancer Res. 32, 2253-2258.

Lee, A.M. and Fraumeni, J.F. (1969). J. Natl. Cancer Inst. 42, 1045-1052.

Lemen, R.A., Lee, J.S., Wagoner, J.K. and Blejer, H.P. (1976). Ann. N.Y. Acad. Sci. 271, 273-279.

Levan, A. (1945). Nature 156, 751-752.

Levy, L.S. and Clark, J. (1975). Ann. Occup. Hyg. 17, 205-211.

Levy, L.S. and Venitt, S. (1975). British Association for Cancer Research, 16th Annual General Meeting and Symposium "Industrial Carcinogenesis." Birmingham, England.

Levy, L.S., Roe, F.J.C., Malcolm, D., Kazantzis, G., Clark, J. and Platt, H.S. (1973). Ann. Occup. Hyg. 16, 111-118.

Levy, L.S., Clark, J. and Roe F.J.C. (1975). Ann. Occup. Hyg. 17, 213-230.

Lilly, L.J. (1975). Toxicology 4, 331-340.

Machle, W. and Gregorius, F. (1948). Publ. Health Rep. Wash. 63, 1114-1127.

Malenkho, A.F. and Bakun, N.A. (1973). Dokl. Akad. BSSR 17, 375-377.

Mancuso, T.F. (1970). Environ. Res. 3, 251-275.

Mancuso, T.F. (1975). Paper presented at the International Conference on Heavy Metals in the Environment, Toronto, Canada, Oct. 27-31.

Mastromatteo, E. (1967). J. Occup. Med. 9, 127-136.

Mathews, C. and Al-Doori, Z. (1976). Mutat. Res. 40, 31-36.

Milham, S. and Strong, T. (1974). Environ. Res. 7, 176-182.

Monti-Bragadin, C., Tamaro, M. and Banfi, E. (1975). Chem. Biol. Interact. 11, 469-472.

Nakamuro, K., Yoshikawa, K., Sayato, Y., Kurata, H., Tonomura, M. and Tonomura, A. (1976). Mutat. Res. 40, 177-183.

Nettesheim, P., Hanna, M.G., Dougherty, D.G., Newell, R.F. and Hellman, A. (1971). J. Natl. Cancer Inst. 47, 1129-1144.

Neubauer, O. (1947). Brit. J. Cancer 1, 192-251.

Norseth, T. (1976). Paper presented at the Symposium on the Origins of Human Cancer, Cold Spring Harbour Laboratory, New York.

Obe, G., Beek, B. and Dudin, G. (1975). Mutat. Res. 29, 283.

Oehlkers, F. (1952). Symposium on Chromosome Breakage, June 9-11, 1952. Supplement to Heredity 6.

Ohsaki, Y., Abe, S., Hamma, Y., Yozawa, K., Kishi, F., Murao, M., Sato, H., Date, F., Kawanchi, F., Kobayanshi, T. and Fujita, I. (1974). J. Jap. Soc. Intern. Med. 63, 1198-1203.

Oppenheim, J.J. and Fishbein, W.N. (1965). Cancer Res. 25, 980-985.

Orgel, A. and Orgel, L.E. (1965). J. Mol. Biol. 14, 453-457.

Osburn, H.S. (1969). South Afr. Med. J. 43, 1307-1312.

Osswald, H. and Goettler, K. (1971). Verb. Dtsch. Ges. Pathol. 55, 289-293.

Paton, G.R. and Allison, A.C. (1972). Mutat. Res. 16, 332-336.

Pedersen, E., Hogelveit, A. and Candersson, A. (1973). Int. J. Cancer 12, 32-41.

Petres, J. and Hagedorn, M. (1977). Environ. Health Perspect. 19 (In press).

Petres, J., Schmid, T., Ulrich, K. and Wolf, U. (1970). Dtsch. Med. Wochenschr. P. 95, 79-80.

Pilinskaya, M.A. (1970). Genetika 6, 157-163.

Pilinskaya, M.A. (1971) Genetika 7, 138-143.

Pinto, S.S. (1977). Environ. Health Perspect. 19 (In press).

Pokrovskaya, L.V. (1973). Gig. Tr. Prof. Zabol. 10, 23-26.

Pokrovskaya, L.V. and Shabynina, N.K. (1973). Gig. Tr. Prof. Zabol. 17, 23-26.

Popper, H. and Thomas, L.B. (1975). Ann. N.Y. Acad. Sci. 246, 172-193.

Potts, C.L. (1965). Ann. Occup. Hyg. 8, 55-61.

Prazmo, W., Balbin, E., Barznowski, H., Ejchart, A. and Putrament, A. (1975). Genet. Res. 26, 21-29.

Putrament, A., Baranowska, H., Ejchart, A. and Prazmo, W. (1975). Mol. Gen. Genet. 140, 339-347.

Ramel, C. (1972a). "Mercury in the Environment." (L. Friberg and J. Vostal, eds) pp 169-181. CRC Press, Cleveland.

Ramel, C. (1972b). 2nd meeting EEMS Zinkovy Castle, Czechoslovakia, p 48 (Abstract).

Reddy, J., Svoboda, D., Azamoff, D. and Dumas, R. (1973). J. Natl. Cancer Inst. 51, 891-903.

Reeves, A.L. (1977). In: "Toxicology of Metals." Volume II, pp 85-109. Environmental Health Research Series, 600/1-77-022, Environmental Protection Agency, Research Triangle Park.

Reeves, A.L.. Deitch, D. and Vorwald, A.J. (1967). Cancer Res. 27, 439-445.

Richmond, H.G. (1959). Brit. Med J. 1, 947-949.

Robinson, T.R. (1974). J. Occup. Med. 16, 601-605.

Roe, F.J.C. (1967). In: "Practical Carcinogenic Hazards from Drugs." (R. Truhaut, ed) pp 105-118. Springer-Verlag, Berlin.

Roe, F.J.C. and Carter, R.L. (1967). Int. J. Cancer 2, 370-380.

Roe, F.J.C., Dukes, C.E., Cameron, K.M., Pugh, R.C.B. and Mitchley, B.C.V. (1964). Brit. J. Cancer 18, 674-681.

Rossman, T., Meyn, M.S. and Troll, W. (1975). Mutat. Res. 30, 157-162.

Roth, F. (1958). Virchows Arch. (Pathol. Anat.) 331, 119-137.

Röhr, G. and Bauchinger, M. (1976). Mutat. Res. 40, 125-130.

Saknyn, A.V. and Shabynina, N.K. (1973). Gig. Tr. Prof. Zabol. 17, 25-28 (In Russian).

Schepers, G.W.H. (1964). Ind. Med. Surg. 33, 1-16.

Schmid, E., Bauchinger, M., Pietruck, S. and Hall, G. (1972). Mutat. Res. 16, 401-406.

Schroeder, H.A. and Balassa, J.J. (1966). J. Chron. Dis. 19, 85-106.

Schwanitz, G., Lehnert, G. and Gebhart, E. (1970). Dtsch Med. Wochenschr. 95, 1636-1641.

Shamberger, R.J., Baughmann, F.F., Kalchert, S.L., Willis, C.E. and Hoffman, G.C. (1973). Proc. Natl. Acad. Sci. 70, 1461-1463.

Shimada, T., Watanabe, T. and Endo, A. (1976). Mutat. Res. 40, 389-395.

Shimada, T., Watanabe, T. and Endo, A. (1975). Teratology 12, 210.

Shiraishi, Y. (1975). Humangenetik 27, 31-44.

Skerfving, S., Hansson, K. and Ryman, N.J. (1974). Environ. Res. 7, 83-98.

Somers, E. and Smith, D.M. (1971). Food Cosmet. Toxicol. 9, 185.

Stich, H.F. and San, R.H.C. (1973). Proc. Soc. Exp. Biol. 142, 155.

Stoltz, D.R., Poirier, L.A., Irving, C.C., Stich, H.F., Weisburger, J.H. and Grice, H.C. (1975). Toxicol. Appl. Pharmacol. 29, 157.

Sunderman, F.W. (1973). Ann. Clin. Lab. Sci. 3, 156-180.

Sunderman, F.W. (1976). Prev. Med. 5, 279-294.

Sunderman, F.W. (1976). In: "Advances in Modern Toxicology." (R.A. Goyer and M.A. Mehlman, eds) Hemisphere Publishing Corp., Washington, D.C.

Sunderman, F.W. and Donnelly, A.S. (1965). Amer. J. Pathol. 46, 1027-1041.

Sunderman, F.W. and Mastromatteo, E. (1975). In: "Nickel." (F.W. Sunderman, F. Coulston, G.L. Eichorn, J.A. Fellows, E. Mastromatteo, H.T. Reno and M.H. Samitz, eds) pp 144-188. National Academy of Sciences, Washington, D.C.

Swierenga, S.H. and Basrur, P.K. (1968). Lab. Invest. 19, 663-674.

Task Group on Air Pollution and Cancer - Risk Assessment Methodology and Epidemiological Evidence. (1978). Report from an International Symposium at the Karolinska Institute, Stockholm, 1977. Environ. Health Perspect. (In press, 1978).

Tokudome, S. and Kuratsune, M. (1976). Int. J. Cancer 17, 310-317.

Tseng, W.P. (1977). Environ. Health Perspect. 19, 109-119.

Tsuchiya, K., Ishinishi, N. and Fowler, B. (1977). In: "Toxicology of Metals." Volume II, pp 30-70. Environmental Health Research Series, 600/1-77-022, Environmental Protection Agency, Research Triangle Park.

Tuovinen, O.H. and Kelly, D.P. (1974). Arch. Microbiol. 95, 153-164.

Umeda, M., Saito, K., Hirose, K. and Saito, M. (1969). Jap. J. Exp. Med. 39, 47-59.

United Nations (1972). Ionizing Radiation Levels and Effects. Vol. 1, Levels. United Nations Scientific Committee Report on the Effects of Atomic Radiation.

Van Esch, G.J. and Kroes, R. (1969). Brit. J. Cancer 23, 765-770.

Venitt, S. and Levy, L.S. (1974). Nature 250, 493-495.

Virtue, J.A. (1972). Can. J. Otolaryngol. 1, 37-42.

Vollman, J. (1940). Schweitz A. Allg. Pathol. Bakteriol. 2, 440-443.

Vorwald, A.J. and Reeves, A.L. (1959). Arch. Ind. Health 19, 190-199.

Watanabe, T., Shimada, T. and Endo, A. (1976). Teratology 14, 259.

Weisburger, J.H. (1976). In: "Chemical Carcinogens." (C.E. Searle, ed) pp 1-23. ACS Monograph 173, American Chemical Society, Washington, D.C.

Weisburger, J.H. and Rall, D.P. (1972). In: "Environment and Cancer." (R.L. Clark, ed) p 437. Williams and Wilkins, Baltimore.

WHO (1974). Assessment of the carcinogenicity and mutagenicity of chemicals. WHO Technical Report Series No. 546.

Williams, G.M., Elliot, J.M. and Weisburger, J.H. (1973). Cancer Res. 33, 606.

Witschi, H.P. (1970). Biochem. J. 120, 623-634.

Yeh, S. and How, S.W. (1963). Inst. Pathol. Natl. Taiwan Univ. 14, 25-73.

Zawirska, B. and Medras, K. (1968). Zentralbl. Allg. Pathol. 111, 1-12.

Zollinger, H.U. (1953). Virchows Arch. Pathol. Anat. 323, 694-710.